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KINETICS OF THE STEADY STATE BACTERIAL CULTURE WITH APPLICATION TO THE ACTIVATED SLUDGE PROCESS

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Rensselaer Polytechnic Institute
Troy, New York

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KINETICS OF THE STEADY STATE BACTERIAL CULTURE WITH APPLICATION TO THE ACTIVATED SLUDGE PROCESS

Final Report

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CHAPTER I - INTRODUCTION

SUMMARY

The original purpose of Project WP-288 was to study kinetics of the activated sludge process. In particular, research was to be initiated to investigate the effects of process loading (weight of organic substrate/weight of active organisms/day) and the volumetric process loading (weight of organic substrate/volume of aerator/day) on the development of activated sludge within the reactor. The activated sludge was to be considered from the standpoint of pure synthesis of organisms and also from the development of accumulation of the total mass resulting from a wide range of loadings. It was a secondary aim of the project to develop relationship between the loading and the rate of substrate removal.

The success of the project depended upon developing an activated sludge culture which would operate at a steady state condition. At steady state the environment of the organisms remains essentially constant over a detention period. Numerous attempts were made to develop an environment which would maintain a relatively steady state condition for an activated sludge system. In no case was it possible to maintain an environment in which the mass of activated sludge would remain constant over an indefinite period of time. In the most successful systems, the activated sludge suspended solids varied as much as 5 percent from one detention period to the next.

Inability to maintain a steady state in an activated sludge system apparently stemmed from a prey-predator relationship. The activated sludge system is predominantly bacterial in nature. In addition, there are, within these systems, a sizeable population of higher forms including protozoa, rotifers, and crustaceans. These higher organism forms prey upon the bacteria causing a fluctuation in the population of both the prey and predators. Because of this condition, it was decided to continue the study using simple substrates and pure bacterial cultures. Since the bacteria are predominant within the activated sludge system, a study of their growth would be applicable to the activated sludge process during sustained operation. It was felt that this approach would be the first phase in the synthesis of mathematical models which would eventually be formulated to represent the growth kinetics of the activated sludge system.

The work conducted under this project will be presented in six chapters:

Chapter I - Introduction
Chapter II - Activated Sludge-Unsteady Conditions
Chapter III - Steady State Bacterial Culture-Synthesis
Chapter IV - Steady State Bacterial Culture-Rate of Synthesis
Chapter V - Steady State Bacterial Culture-Carbon Transfer Rates
Chapter VI - Conclusions

These studies revealed that the effective yield coefficient is not a constant for a given system of bacterium and organic substrate. Rather, the effective yield coefficient is a variable function of the true yield coefficient and the hetrogenous metabolism of the organisms. A true yield coefficient was found to be constant for each system of bacterium and organic substrate but varied widely between the various systems. A true yield in a mixed culture system composed of two or more bacteria on a given organic substrate was determined to give a higher true yield coefficient than with the corresponding pure culture system.

The rate of growth of the bacteria in the systems studied showed a uniform variation with the concentration of the organic substrate. Although not conclusive, the data tends to support mathematical relationship similar to the Michaelis-Menton equation for enzyme activity. In order to make the equation relating growth rate to substrate concentration applicable to present engineering practice, a correction in the formula was needed to account for the gross measure of substrate rather than the removal rate of the specific substrate. A relationship was developed using chemical oxygen demands as a gross measure of the organic substrate.

To enable a better understanding of the growth kinetics of bacteria in continuous culture, a mathematical compartmental system was developed. The resulting equations related the transfer rates of carbon between the various sources and sinks within the system. To obtain a measure of the transfer rates at various process loadings (dilution rates), steady state cultures were inoculated with uniformly labeled Carbon-14 organic substrates. The transfer rate equations were then solved by determining analytically the carbon contents of the various compartments and the total activity accumulated in these compartments by radio assay. This radio tracer technique proved to be most useful in the study of bacterial kinetics. The data obtained from the radio assay and Van Slyke carbon analyses were unusually consistent for biological systems. This system of analysis enabled the determination of a maximum yield occurring as a function of the dilution rate. This maximum effective yield occurred at a dilution rate of approximately 0.5 hydraulic turnovers per hour. The yield was observed to decrease at dilutions greater and also less than this value. An analysis of the transfer rates showed that the maximum which occurred in the effective yield was mainly the result of a minimum which occurred in the discharge of organic material from bacterial culture into the surrounding media. Although the occurrence of this maximum effective yield has been shown by other investigators, the data found here lead to a different hypotheses as to the cause of this maximum condition.

This research has resulted in the development of kinetic equations for the growth of bacteria in a steady state culture as well as equations for the removal of substrate associated with synthesis. Although the coefficients for these equations are specific to a given system of bacteria and organic substrate, the form of the equations is generally applicable to any predominantly bacterial system. The equations have gone beyond those generally presented by bacteriologists in that they include a term for hetrogenous metabolism, i.e. the endogenous metabolism of the viable cells and the degradation of the products of autolysis. This phenomenon is significant in those biological processes which operate at a high active mass level over long detention periods. Previous equations developed for substrate removal have been improved by including a consideration of the energy metabolism coefficient. This coefficient takes into consideration the removal of substrate from the media without accompanying synthesis for the purpose of maintaining a basic energy requirement of the organisms. It is felt that the equations which have been developed are generally applicable to the activated sludge process. The coefficients would have to be determined for a given waste stream. Because of the variable nature of the activated sludge system, it is expected that the coefficients would be mean values with standard deviations determined from numerous analyses of the system. It is, therefore, evident that the results of this project are not a solution to the formulation of kinetics of activated sludge but rather the development of the basic equations. It is hoped that as stochastic relations between the prey and the predators of the activated sludge are better understood that a more precise measure of the variables in the deterministic equations for activated sludge can be evaluated.

OBJECTIVE

The objective of this research was to evaluate the existing equation for mass production and substrate removal in an activated sludge process based upon the response of laboratory pilot systems. Upon evaluation, the equations were to be modified or discarded in favor of new equations which would better represent the experimental results. Finally, the applicability of the model for the experimental system to the field activated sludge process was to be estimated.

This project did not attempt to include a full evaluation of the mathematical models to the design and control of field installations. Such an extensive field study as would be required was best suited for a future project.

Symbols

All symbols used in this report are defined at the point of their initial use. Also, the complete nomenclature is given in Appendix A.

CHAPTER II - ACTIVATED SLUDGE

UNSTEADY CONDITIONS

INTRODUCTION

Prior to the initiation of this research, essentially all development of deterministic equations relating substrate removal and mass accumulation with time in an activated sludge system had been hypothesized based upon analyses of model or prototype activated sludge units. It was felt that these formulae represented a gross oversimplification of the actual growth kinetics. Fully realizing that the engineer's responsibility is to generalize on complex systems to enable their design, the generalized equations currently under use showed evidence of lacking correlation to field experience. One of the primary causes for this might well be the failure of the model to consider the importance of certain variables.

To study the kinetics of the growth of activated sludge, laboratory model systems were used. These models had hydraulic and biological similitude to the prototype process. The organic substrates used were, however, of simple organic structure and were applied at a constant loading rate. This was done to reduce the fluctuations in the system which would occur as a result of variable substrate quality and quantity normally associated with sewage discharges.

This chapter contains the report of the attempt to use model activated sludge systems to study the detailed kinetics of that process. Although unsuccessful in its aims, this portion of the research was a necessary preliminary step in the research program.

It showed that the effect of prey-predator activity was large enough to mask important factors in the activity of the bacterial population.

KINETICS OF SLUDGE ACCUMULATION

A number of laboratory studies have been conducted by Environmental Engineering researchers in order to determine the expected sludge accumulation in activated sludge plants operating under various conditions. Some of the researchers have attempted to mathematically formulate the expected accumulation as a function of what they felt to be the primary variables. The removal of substrate has, for the most part, been considered indirectly as a necessary preliminary step toward synthesis. In preparation of a discussion of the results of this research, some of the more noteworthy research of the past will be discussed.

The first comprehensive effort to correlate the factors affecting the quantity of sludge production in the activated sludge process was presented by Heukelekian, Oxford and Manganelli(23). They found a scarcity of information in the literature on the subject of synthesis of activated sludge. Of the few available articles, a large percentage gave insufficient information to allow a thorough evaluation of the data. For their study they used five different sources of sewage, largely domestic in origin, most of which had appreciable amounts of suspended solids in relation to their BOD. Batch units were used with a detention time of approximately eight hours. Each sewage was fed to a unit maintained at three mixed liquor suspended solids concentrations (MLSS). To obtain the necessary information to determine the sludge solids accumulation, routine analyses were made on the sewage, suspended solids, volatile solids and BOD, also, total and volatile suspended solids of the mixed liquor.

The results showed an inverse relationship between suspended solids in the mixed liquor and the sludge buildup. This relationship, they felt, was better defined between sludge age, (i.e. lb. MLSS/lb. suspended solids fed/day) and lb. suspended volatile solids accumulated/lb. suspended solids fed/day. The data from all their tests together with that from Sawyer's(59) investigation follows the growth equation:

$$P_v = -0.055M + 0.5S_{1B}\frac{Q}{V} \qquad (2\text{-}1)$$

In this expression the production of volatile solids per volume of reactor per day, P_v, is decreased by a constant proportion of the mass as volatile solids and increased by a constant proportion of the substrate concentration in the influent as 5-day BOD, S_{1B}, times the daily discharge divided by the volume of the reactor. The equation, as given by Heukelekian, related the weight of cells grown to the weight of cells present and the 5-day BOD of the substrate. By substituting in the more recently used term of volumetric process loading L_5 (wt. 5-day BOD/volume-day), Equation (2-1) becomes

$$P_v = 0.5L_5 - 0.055M \qquad (2\text{-}2)$$

The authors felt that, at least within the precision of Environmental Engineering Design, the production of volatile solids was independent of the process loading, i.e. weight of substrate applied per weight of active organism per day, over the range of process loading of 0.14 to 1.0 lb. BOD/(day-lb. M). The sludge produced P_v could well be accumulated A_v in the reactor or wasted from the system.

Subsequent research by the same group [Gellman and Heukelekian(14)] on the oxidation and removal rates of different industrial wastes with normal and acclimated activated sludge, brought somewhat different results. These soluble wastes, jute cook liquor, yeast waste, gum waste, and boardmill white water, were all found to yield about 0.5 lb. of volatile solids in new sludge growth per lb. of BOD fed. The data gave no apparent divergence from a straight line relationship over a wide range of process loading (0.6 to 6.0 lb. BOD/day-lb. M).

These latter results are supported by the work of both Greenberg(16) and Helmers(20), who concluded that the growth rate per day in activated sludge was proportional to the BOD fed. Greenberg working with brewery waste found a conversion of about 54 percent of the BOD to sludge over a process loading range of from 0.5 to 1.7 lb. BOD/(day-lb. M). Helmers working with cotton kiering waste measured about the same conversion factor with process loading from 0.3 to 1.1 lb. BOD/(day-lb. M). On considering the apparent conflict between the results of Heulelekian(23)(59), Sawyer(60) suggested that the high suspended solids in the sewage had caused the reduction in the percent synthesis with increased sludge production. He further stated that synthesis could be predicted from the BOD of a waste by the simple formula

$$P_V = 0.5 F_{AB} \tag{2-3}$$

where F_{AB} is the food applied in terms of the BOD. In light of more recent work on the nature of the 5-day BOD by A. W. Busch at Rice University(4), Equation (2-3) appears to be a gross oversimplification of the kinetics of activated sludge growth.

Gram(15) studied the kinetics of aerobic biological processes. He theorized that the growth in an activated sludge system follows the equation:

$$\frac{A_T}{M_T} = -K_2' + \frac{BL_5}{M_T} \qquad (2\text{-}4)$$

where A_T represents the total suspended solids accumulation per day. Therefore, A_T/M_T is the fractional increase in solids. K_2' is the hetrogenous metabolism rate based upon total suspended solids and B is the yield coefficient based upon the gross measure of 5-day BOD. His research was directed toward justifying his equation and determining the coefficients. For an activated sludge system fed skim milk in the process loading range of 0.2 to 1.1 lb. BOD/(day-lb. M_T), the rate of sludge accumulation followed the equation:

$$\frac{A_T}{M_T} = -0.045 + 0.53\,\frac{L_5}{M_T} \qquad (2\text{-}4a)$$

It can be seen that if Equation (2-4) is multiplied by M_T it becomes identical in form to Equation (2-1).

Weston and Eckenfelder(10)(75) showed that the experimental results on sludge accumulation of others (9)(24)(52) indicated a definite relation existed between amount of BOD removed, the portion oxidized, and the portion synthesized for any given set of conditions. In practice, the relation was difficult to determine due to concurrent oxidation of the cell material.

Dryden, et al.(9) reported an equation for calculating sludge accumulation in a high-rate activated sludge treatment process for fine chemical wastes. Later Eckenfelder and Porges(11) generalized the formulation.

$$VP_v = BF_{RB} - K_2MV \qquad (4\text{-}5)$$

where F_{RB} is the food removed as BOD and V is the system volume. This equation deals with the total sludge production in the system. To be compared with the formulas of other workers it can be converted to sludge production per tank volume P_v by dividing through by the tank volume.

$$P_v = \frac{BF_{RB}}{V} - K_2M \qquad (2\text{-}5a)$$

Equation (2-5a) is similar to that given by Heukelekian(23), Equation (2-1), except for the refinement of using the BOD removed rather than the BOD applied. Eckenfelder and Porges(11) took this approach because of their belief that for high volumetric process loadings, the removal approaches a limiting rate. The system, they felt, was then in log growth (maximum growth) phase where growth rate was a function of the cell generation time.

Since the inception of this project, numerous articles on the kinetics of activated sludge have appeared in the literature. These reports will not be considered here, for this discussion concerns the status of the subject prior to the initiation of this research program. Reviews of the recent progress in this area have been incorporated into articles by Servizi(62) and Busch(4).

EXPERIMENTAL PROCEDURES

The laboratory activated sludge systems were designed to reproduce the biological activity in a homogeneous reactor with sludge recycle. The model complete mixing activated sludge units were constructed of plexiglass with liquid capacity of 1.5 liters. A baffle was placed such as to divide the compartment into an aeration section and an upflow settling section, with the former being twice the capacity of the latter.

Air was supplied through a porous diffuser in the lower back wall of the aeration section. The pressure of the air supply was maintained at 15 psig during the operation of the systems. The hydraulic lift of the influent air bubbles produced a rolling effect similar to that in the prototype. Thus, the mixed liquor was completely mixed during aeration. The quantity of air was maintained at 1 liter per liter of aeration capacity per minute which was more than sufficient to keep the aeration solids in turbulent suspension. The units were fed continuously through a tube in the back of the compartment. The feed was metered by a Harvard type proportional pump. As a portion of substrate solution entered the unit and was completely mixed with the content of the aeration section, an equal volume of mixed liquor was displaced to the upflow settling section. In the settling section, the suspended solids were settled and the supernatant liquid was displaced into an effluent container.

The organic substrates chosen for this study were glucose and glutamic acid. The substrate solution was prepared with Troy tap water in which the organic substrate was dissolved. In addition, sufficient nitrogen was added to the feed solutions to insure a fifty percent excess over that required for eighty percent conversion of the substrate to synthesis. The nitrogen was added as ammonium nitrate. An abundance of phosphorus was added in the form of phosphate buffer. All systems received 1.0 gram of pH = 7.0 potassium phosphate buffer per day. In all systems studied, the volumetric loading was 0.5 gm/liter/day or approximately 30 lb/1,000

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cu. ft./day. The process loading was varied by control of activated sludge wasting.

The activated sludge was obtained from a local extended aeration activated sludge plant. The mixed liquor volatile suspended solids (MLVS) at the plant averaged about 3,000 mg/l. Prior to their inoculation into the laboratory units, the MLVS were measured and the sludge diluted with dechlorinated tap water. The diluting tap water was at room temperature.

The suspended solids carried in the system were controlled by continuous sludge wasting. Since the metabolic activity and consequently the kinetics of substrate removal and mass growth are dependent upon the process loading, it was most vital to the aims of the research that a constant MLVS level be maintained in the reactor. The MLVS were taken daily to enable a daily check on the solids level in the system and, more important, to enable an adjustment of the wasting rate of the MLVS. The MLVS sample was taken daily at noon. This represented the MLVS at the end of the daily feed period. Based upon these results the solids level and mixed liquor wasting rates were adjusted every day. The mixed liquor was wasted rather than the settled solids because more uniform removal could be accomplished in this manner.

The suspended biological solids were determined by the membrane filter method as described by Engelbrecht and McKinney(13). Unless otherwise noted, the aeration solids samples were 20 ml. These samples were taken after thorough mixing of the entire tank contents. The effluent samples were as large as practicably filterable, which, depending upon the prevailing conditions, varied from

50 to 500 ml. To measure the ash content, the crucible, after weighing with the dried sludge, was covered and ignited at 600°C for one hour. The curcible was then cooled and weighed. The biological volatile suspended solids, from the mixed liquor (MLVS) or the effluent, were taken as the difference between the total biological solids and the inorganic ash.

The soluble chemical oxygen demand (COD) was determined by a modification of the procedure given in Standard Methods(1). The modified method used one-fifth the suggested volume of the sample (10 ml) and reagents. The silver sulfate catalyst was used in all the determinations.

RESULTS AND DISCUSSION

Preliminary runs were made with systems using either of the organic substrates. All these cultures were operated at a process loading of 0.25 lbs. COD/lg. MLVS which is similar to that employed in conventional activated sludge installations. The systems grown on each of the substrates will be considered separately.

Glucose System - The system which used glucose as the sole organic carbon source was operated for an eight week period. It was intended that the volatile solids level would be maintained at 2,000 mg/l. As shown in Figure (2-1), the weekly average MLVS values show a fairly consistent steady-state condition with an average MLVS of about 2,100 mg/l. Most all the weekly average values for MLVS fell within ± 5 percent of the mean. It should be noted that the weekly average production of volatile solids varied markedly. Most disturbing was the large variation in the daily MLVS in the reactor. The MLVS, as sampled at noon daily, varied

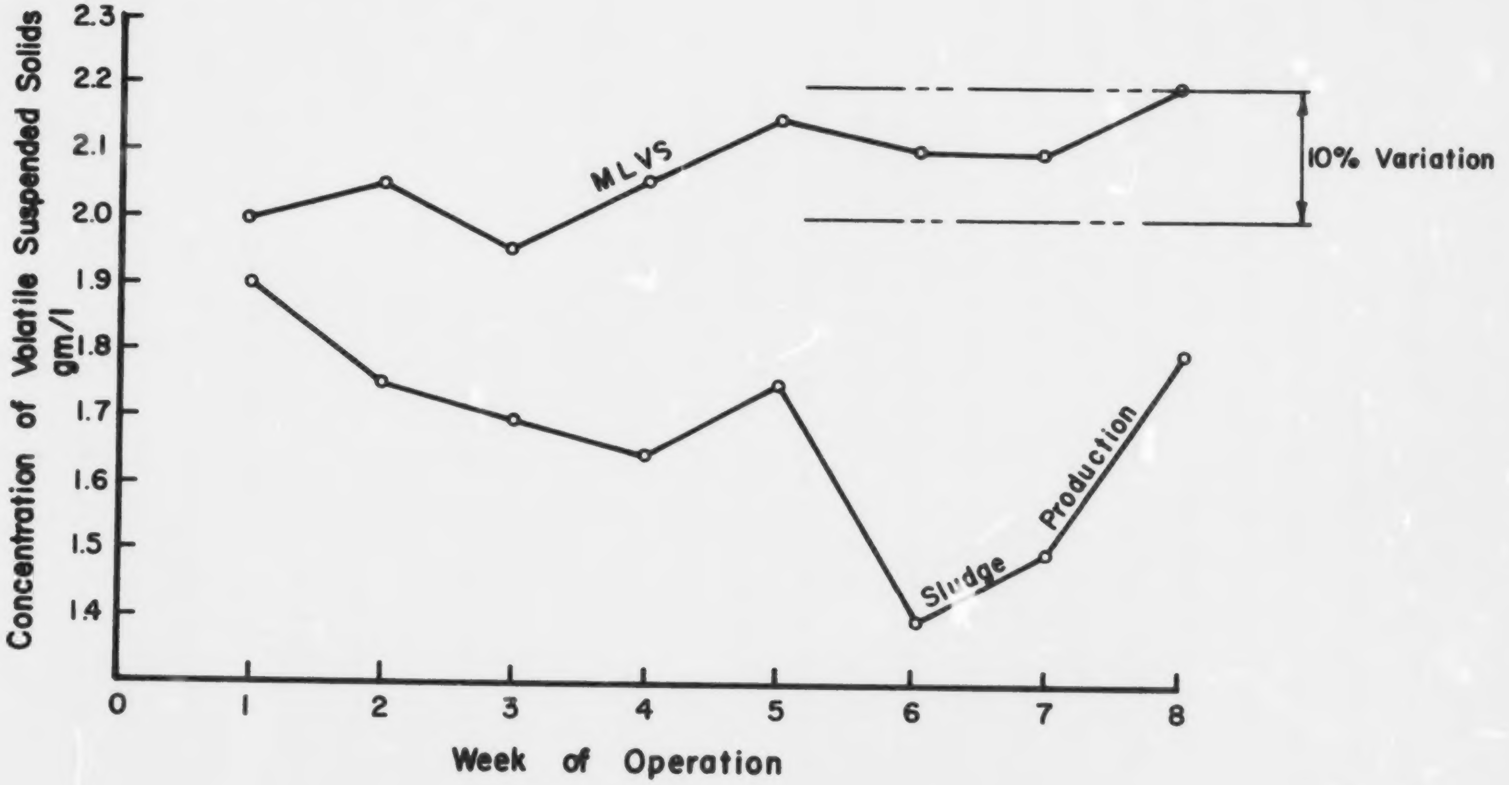

FIGURE 2-1 – SOLIDS CARRIED IN GLUCOSE FED ACTIVATED SLUDGE SYSTEM

from 1,850 to 2,300 mg/l. This represents a spread of about 20 percent during the course of the study. This large variation in the system activated sludge solids is graphically shown for the fourth week of the study in Figure (2-2).

Several functional difficulties were encountered which most likely contributed to the large variations in the solids level. The first definable problem occurred during the second week when microscopic examination revealed a sudden growth of fungi in the activated sludge culture. The fungi never gained predominance but were present in sizeable numbers. This situation was remedied by feeding the system without aeration for 24 hours. The normal operational procedure was then resumed. Within two days, the flora and fauna appeared normal for an activated sludge culture.

In the seventh week, sludge rising in the settling compartment became a serious problem. This trouble arose from improper circulation of the sludge in the settling compartment. The resulting anaerobic conditions caused denitrification in the sludge.

Glutamic Acid System - The system which used glutamic acid as the sole organic carbon source was operated for a ten week period. It was again intended that the volatile solids level be maintained at about 2,000 mg/l. As shown in Figure (2-3), the weekly average MLVS values show an apparent steady-state condition at 2,025 mg/l. The weekly average MLVS values fell within ± 5 percent of the mean. The total sludge production was more consistent than in the glucose system.

Here again, it was not possible to maintain a steady solids level in the reactor. The mid-day solids analyses showed a

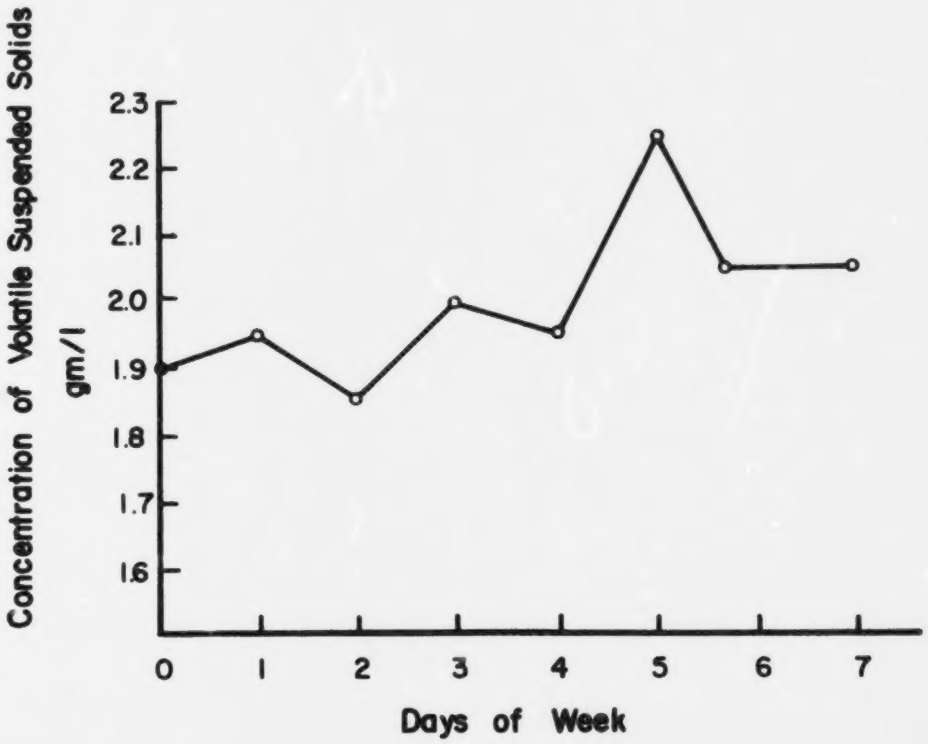

FIGURE 2-2 — VOLATILE SOLIDS IN ACTIVATED SLUDGE SYSTEM DURING THE THIRD WEEK

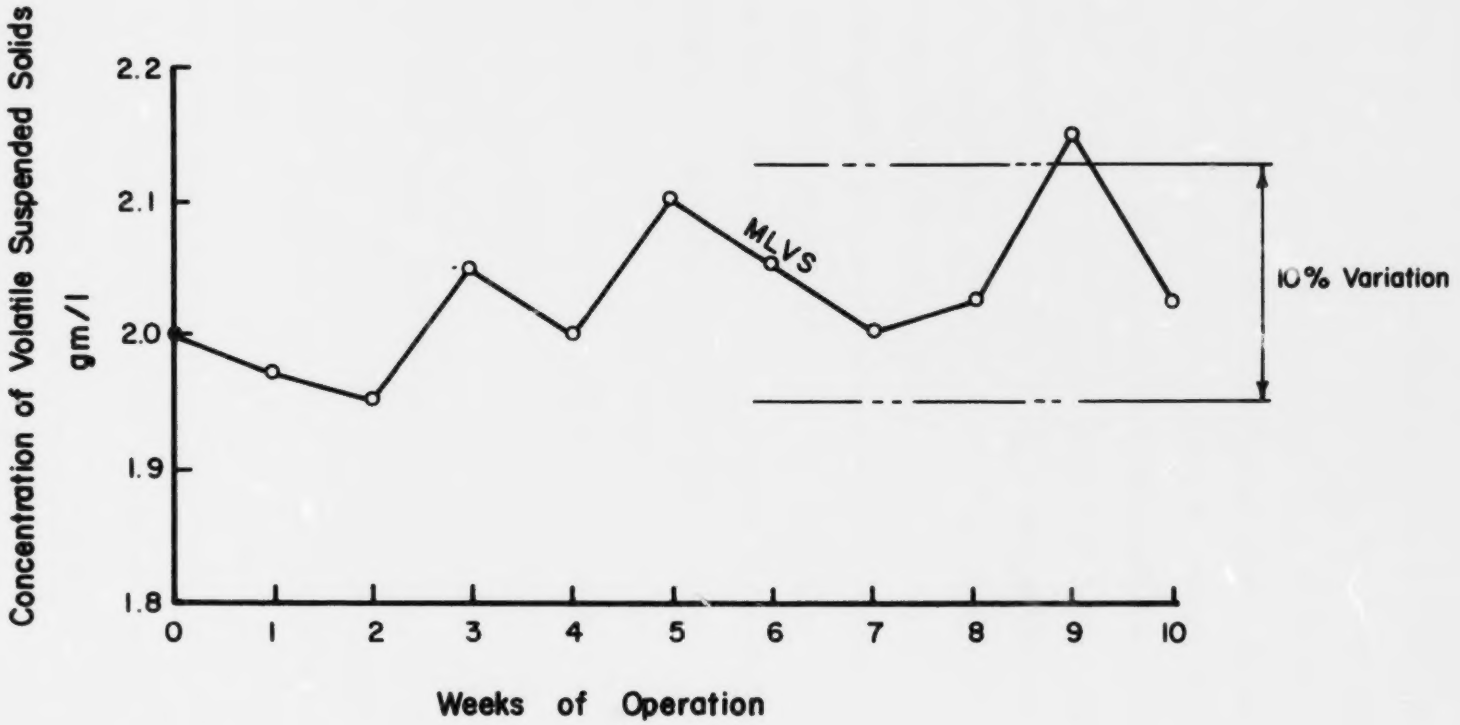

FIGURE 2-3 — SOLIDS CARRIED IN GLUTAMIC ACID FED ACTIVATED SLUDGE SYSTEM

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variation of MLVS from 1,950 to 2,150 mg/l over the period of study. This represents a 10 percent variation in the daily solids level in the reactor.

The flora and fauna in this system was normal through the investigation. However, denitrification was more serious in this system than in the glucose system. The solids lost as a result of denitrification were varied but some losses occurred during each of the ten weeks of the study.

It was felt that a good part of the fluctuation of solids level in both the systems studied was the result of prey-predator activity. In a mixed culture system, the bacteria population increased to a maximum value depending upon the total substrate available. Once at this point, the MLVS due to bacteria would stay approximately steady if they were not subject to metabolism by higher forms. In the bacteria-rich environment, the predators tend to increase at the expense of the prey. Once the prey level is decreased below a threshold value the predator begins to die out. This, once again, allows the bacteria to increase in numbers and the cycle begins again.

Because of the observed difficulties, it was decided that a more stable culture would be required for the study of the growth kinetics of activated sludge. It was agreed that cultures of pure bacteria should be studied in order to obtain deterministic equations for the major organism in activated sludge. It was realized that the results would not be directly applicable to activated sludge process design, but rather the initial step to a delineation of the kinetics of activated sludge.

CHAPTER III - STEADY STATE BACTERIAL CULTURE SYNTHESIS

INTRODUCTION

This chapter and Chapter IV show how and to what extent the kinetics of the bacteria in the steady state can be determined using conventional chemical and biological analytical techniques. Further, they discuss how the findings can be applied to the activated sludge process.

The continuous culture of bacteria at a steady-state eliminated many of the disadvantages of the mixed culture activated sludge. Maintaining a reproducible steady-state environment in the system enabled the determination of environmental factors with replicate measures. Full advantage was taken of the ability of the system to maintain any desired environment. The results of these analyses are considered here. However, as will be seen later, the constant environment made determination of the rate of reactions difficult or impossible

THEORETICAL CONSIDERATION

The modern theory of the growth of bacteria in continuous culture was started by Monod in the early 1940's(41). This work on continuous culture kinetics was furthered by Maxon(33) and Novick(47). Herbert, et al. presented a classical paper on the subject in 1956(22). However, all these workers are interested in relatively short term fermentations compared to those used in the waste treatment industry. As a consequence, their models give little consideration to the autolysis which seems of importance in the waste treatment field. Based upon the present theory for pure culture growth and that for activated sludge, equations were developed to represent the mass and substrate balance in the homogeneous, steady-state bacterial culture without culture recirculation.

Mass Balance

The rate of change of mass or organisms in the reactor can be described by the balance equation (individual terms are rates)

mass change = growth - loss in effluent - death

or

$$\frac{dM_a}{dt} = \mu M - DM_a - f(S_c, M_a) \qquad (3\text{-}1)$$

where M_a is the active organism concentration in the culture vessel at time, t, μ is the specific growth rate, D is the dilution rate equal to flow rate divided by volume of the reactor (V) and S_c is the concentration of organic material in solution in the reactor at time, t.

The function describing the "death" contribution has been discussed by Moser(42) and Powell(54) and is generally taken to be merely some function of the mass of organisms present, even though it is probably a function of the concentration of substrate (S_c) as well. The death rate of the organisms could be effected by both toxic materials in the influent and toxic end products. For the development of this model, it was assumed that toxic materials were not present in sufficient quantities to effect the system. The coefficient for the "death" contribution herein called the hetrogenous metabolism coefficient, K_2, is the result of endogenous metabolism and metabolism of autolysis products. Under these assumptions, equation (3-1) becomes

$$\frac{dM_a}{dt} = \mu M - DM_a - K_2M_a \qquad (3\text{-}1a)$$

The effective growth rate can be defined as

$$\mu_e = \mu - K \qquad (3\text{-}1b)$$

and equation (3-1a) thus becomes

$$\frac{dM_a}{dt} = \mu_e M_a - DM_a \qquad (3\text{-}1c)$$

Substrate Balance

The rate of change of amount of substrate in the culture vessel is described by the following balance or conservation equation (individual terms are rates again)

$$\begin{matrix}\text{substrate} \\ \text{change}\end{matrix} = \text{input} - \text{output} = \begin{matrix}\text{use by} \\ \text{organisms}\end{matrix} - \begin{matrix}\text{amount contributed} \\ \text{by lysis and death}\end{matrix}$$

or

$$\frac{dS_c}{dt} = DS_i - DS_c + G(S_c, M_a) + pqf(S_c, M_a) \qquad (3\text{-}2)$$

where S_i is the concentration of substrate in the incoming feed, S_c is the concentration of substrate in the reactor, p is fraction of cells which die that lyse and q is fraction of substrate released per unit of cells lysed.

The rate of change of substrate (S_c) due to removal by growing microorganisms, has been taken to be directly proportional to cell growth by most workers (Herbert(22), Maxon(33)). The proportionality constant can be considered as the reciprocal of the measured or observed yield coefficient (Y). A better yield coefficient (Z) on the other hand is defined as weight of organisms synthesized per weight of substrate used for this synthesis.

Besides using substrate for growth, most microbiologists (7)(36)(46) conclude that bacteria consume substrate to provide energy for purposes not directly related to growth. This energy is hypothesized as being expended to maintain different concentrations of solute across cell membranes and to provide the energy required for resynthesis of the macromolecules which are breaking down because of the hydrolysis of proteins and nucleic acids to their constituent monomers and to provide energy for motion.

In the absence of an exogenous substrate to supply this required energy the organism will metabolize some part of their own protoplasm to provide it. This use of cell protoplasm, to provide for an energy of maintenance in the absence of an exogenous substrate, has been called endogenous metabolism by microbiologists(30).

In the presence of an exogenous substrate the bacteria still requires this energy for maintenance but it can now be supplied by the exogenous substrate.

The rate of substrate taken up by bacteria to provide for this energy can be called an energy metabolism rate and written as:

$$\frac{dpS_c}{dpt} = K_1 M_a \tag{3-3}$$

where $\frac{dpS_c}{dpt}$ is the rate of uptake of substrate in mg/l/hr required to provide for the energy metabolism requirements of the active organism M_a (mg/l) with a specific energy metabolism rate K_1 (mg substrate/mg cell/hr).

As in equation (3-1a) the death function, $f(S_c, M_a)$ can be taken as K_2M_a. Then equation (3-2) may be expressed as

$$\frac{dS_c}{dt} = DS_1 - DS_c = \frac{\mu M_a}{Z} - K_1M_a + pqK_2M_a \qquad (3\text{-}2a)$$

where Y is usually less than Z as a result of $f(K_1)$, a function describing the uptake of substrate for energy metabolism.

Little or no work has been done on the value of p and q which appear in equation (3-2a). An approximate maximum value of the product p x q has been obtained from the literature (Postgate (53)) as

$$pq = 0.004$$

This value was obtained by assuming a cryptic growth value of one new organism for 500 dead ones and a yield of fifty percent. Since K_2 is also a fraction less than one, the assumption was made in working with a pure bacterial culture on a single substrate that the last term of equation (3-2a) can be dropped without significantly changing the result. This assumption would probably not be valid in the case of recirculation, e.g. activated sludge or any industrial fermentation process which involves recirculation and/or long sludge retention periods. (As will be shown in Chapter V these assumptions which allowed the substrate contribution from the cells to be ignored was incorrect.)

Assuming that the fourth term of equation (3-2a) is negligible, then the substrate removal for synthesis would all be derived from the influent organic nutrient. Under this assumption, the yield coefficient (Z) equals the yield coefficient (C), i.e. the synthesis of active mass resulting from the substrate removed for synthesis. The change in substrate from equation (3-2a) then becomes

$$\frac{d\ S_c}{dt} = DS_i - DS_c - \frac{\mu M_a}{C} - K_1 M_a \qquad (3\text{-}2b)$$

Effective Yield

The effective loss of substrate due to cell growth and energy metabolism may be defined in terms of Y as

$$\frac{\mu_e}{Y} = (\frac{\mu}{C} + K_1) \qquad (3\text{-}4)$$

substituting into equation (3-2b)

$$\frac{d\ S_c}{dt} = DS_i - DS_c - \frac{\mu_e\ M_a}{Y} \qquad (3\text{-}2c)$$

Solving equations (3-1c) and (3-2c) for the steady state, i.e.

$$\frac{dM_a}{dt} = \frac{dS_c}{dt} = 0,$$

results in an expression for the effective yield coefficient

$$Y = \frac{(\mu - K_2)M_a}{DS_i - DS_1} = \frac{\mu_e\ M_a}{D(S_i - S_1)} \qquad (3\text{-}5)$$

This relationship between the quantity of cell material synthesized and the amount of substrate utilized, having the units of weight of cells produced per weight of substrate removed, is usually expressed for comparing the protoplasm production in biological systems.

The effect of energy metabolism on the effective yield coefficient may better be seen by consideration of material balance equations when K_1 remains in the expression. From Equation (3-1a) at a steady state, it is evident that

$$\mu = D + K_2 \qquad (3\text{-}6)$$

Combining Equations (3-2b) and (3-6)

$$0 = D(S_i - S_c) - \frac{DM_a}{C} - \frac{K_2M_a}{C} - K_1M_a, \qquad (3\text{-}7)$$

dividing by the substrate removal, $D(S_i - S_c)$, yields

$$1 = \frac{K_2}{DC}\left(\frac{M_a}{S_i - S_c}\right) - \frac{1}{C}\left(\frac{M_a}{S_i - S_c}\right) - \frac{K_1}{D}\left(\frac{M_a}{S_i - S_c}\right), \qquad (3\text{-}8)$$

$$1 = \left(\frac{M_a}{S_i - S_c}\right) \quad \frac{K_2}{DC} + \frac{1}{C} + \frac{K_1}{D} \quad , \qquad (3\text{-}9)$$

$$\frac{M_a}{S_i - S_c} = \frac{1}{\frac{K_2}{DC} + \frac{1}{C} + \frac{K_1}{D}} \qquad (3\text{-}10)$$

As was indicated earlier, most previous experimental measurements of the yield coefficient neglected the effect of the hetrogenous metabolism and energy metabolism (assumed K_1 and $K_2 = 0$) and observed a yield coefficient

$$Y = \frac{\mu_e \; M_a}{D(S_i - S_c)} \qquad (3\text{-}5)$$

But combining Equations (3-10) and (3-5) the observed yield coefficient is actually

$$Y = \frac{1}{\frac{K_2}{DC} + \frac{1}{C} + \frac{K_1}{D}} \qquad (3\text{-}11)$$

Since the dilution rate, D, is equal to the reciprocal of the detention time, T_d,

$$Y = \frac{1}{\frac{K_2T_d}{C} + \frac{1}{C} + K_1T_d} \qquad (3\text{-}12)$$

Equation (3-12) shows that the observed yield coefficient, Y, will only be equal to the true yield coefficient, D, when the detention time, T_d, is equal to zero. If T_d has any value greater than zero, the observed yield coefficient will be smaller than the true yield coefficient. Since T_d cannot be zero for any measurement, it is not possible to measure C directly.

Equation (3-13), however, can be transformed to

$$1/Y = \frac{1}{C} + \left(\frac{K_2}{C} + K_1\right) T_d \qquad (3\text{-}13)$$

which is a straight line of the type $y = b + ax$ where $b = \frac{1}{C}$ and $a = (\frac{K_2}{C} + K_1)$. It is then possible to obtain C by measuring the yield coefficient, Y, at various dilution rates and plotting 1/Y vs T_d. Extrapolation of the line to the ordinate gives the value of 1/C and C can be determined.

Laboratory research reported in this chapter was conducted to evaluate the various coefficiencies discussed above. The significance of these equations and coefficiencies is obviously dependent on the validity of the assmuptions made in their derivation. The assumptions were considered consistent with good judgement and justified in order to make some initial improvements on the kinetics of bacterial growth. It is felt that the approach used is applicable in principal to obtaining data for improved engineering design of activated sludge facilities.

VARIATIONS OF THE EFFECTIVE YIELD COEFFICIENT WITH SUBSTRATE AND ORGANISM

Experimentally, engineers and biologists have measured the effective yield coefficient by means of a material balance relating the accumulation of cell material to the removal of substrate under batch growth conditions. A tabulation of values found in this manner and reported in the biological and engineering literature are shown for pure culture in Table (3-1) and for mixed culture in Table (3-2). These tables show a significant spread in the reported values of the effective yield coefficient, even for the same substrate, when measured by different investigations.

The relationship between the effective yield coefficient and the nature of the organic substrate metabolized is an area in which there has been considerable speculation and confusion both in the biological and engineering literature.

In the biological literature, most of the published work on this topic indicates that a stoichiometric relationship exists between the quantity of substrate oxidized and the quantity used in cell synthesis for a given organism and a given compound under similar conditions. Many attempts to relate the synthesis to the change in the free energy (ΔF) of the substrate were made but no experimental evidence could be found to support this relationship. In reviewing the subject, Lamanna and Mallette(28) conclude that the chemical nature of a substrate rather than its change in free energy controls synthesis during growth.

TABLE (3-1)

Reported Effective Yield Coefficients for Pure Cultures

Substrate	Organism	Growth Conditions	Reported Yield		References
			mg cells/ mg substrate	mg cells/ mg substrate COD	
Acetate	Purple Bacteria	Batch	0.36	0.34	42
Arabinose	*E. coli*	Batch	0.24	0.22	41
Arabinose	*E. coli*	Batch	0.51	0.48	63
Fructose	*E. coli*	Batch	0.22	0.18	41
Fumarate	*E. coli*	Batch	0.36	0.44	63
Glucose	*A. aerogenes*	Batch	0.32	0.30	57
Glucose	*A. aerogenes*	Continuous	0.44	0.41	57
Glucose	*E. coli*	Batch	0.47	0.44	63
Glucose	*E. coli*	Batch	0.24	0.23	41
Glycerol	*A. cloacae*	Continuous	0.52	0.44	22
Glycerol	*E. coli*	Batch	0.51	0.42	63
Glycerol	*A. cloacae*	Batch	0.53	0.43	22
Lactate	*Spirillum serpens*	Continuous	0.42	0.39	26
Lactate	*E. coli*	Batch	0.32	0.30	63
Lactose	*E. coli*	Batch	0.44	0.41	63
Pyruvate	*E. coli*	Batch	0.36	0.40	63
Rhamnose	*E. coli*	Batch	0.16	0.14	41
Succinate	*Pseudomonada KB-1*	Continuous	0.27	0.18	57
Succinate	*E. coli*	Batch	0.33	0.22	63
Xylose	*E. coli*	Batch	0.21	0.20	41

TABLE (3-2)

Reported Effective Yield Coefficients for Mixed Cultures*

Substrate	Reported Yields mg cells/ mg substrate	 mg cells/ mg substrate COD	References
Acetic Acid	0.38	0.36	37**
Acetic Acid	0.34	0.32	52
Acetic Acid	0.36	0.34	62
Alanine	0.45	0.31	62
Alanine	0.47	0.44	37**
Anthranilic Acid	0.54	0.33	62
Arabinose	0.61	0.57	37**
Benzoic Acid	0.87	0.44	37**
Benzoic Acid	0.56	0.33	62
Brewery Wastes	--	0.36 to 0.41	60
Brewery Wastes	--	0.63	12
Butyric Acid	0.74	0.41	37**
Citric Acid	0.40	0.53	37**
Citric Acid	0.29	0.39	62
Cotton Kiering Liquor	--	0.36 to 0.41	60
Dextrin	0.49	0.41	52
Dextrin	0.59	0.50	52
Ethanol	0.46	0.22	14
Ethyl Alcohol	0.33	0.16	52
Formic Acid	0.17	0.50	37**

TABLE (3-2)

(Cont)

Substrate	Reported Yields mg cells/ mg substrate	mg cells/ mg substrate COD	References
Fructose	0.67	0.55	37**
Fumoris Acid	1.10	0.57	37**
Glucose	0.62	0.58	37**
Glucose	0.40	0.38	62
Glutamic Acid	0.40	0.53	37**
Glutamic Acid	0.35	0.36	62
Glycerol	0.46	0.38	62
Glycine	0.26	0.20	37**
Heptanoic Acid	0.01	0.39	37**
Hydrocinnamic Acid	0.68	0.35	62
Iso Propanol	0.14	0.06	14
Lactose	0.83	0.78	52
Lactose	0.37	0.35	24
Malic Acid	0.38	0.53	37**
Maltose	0.38	0.36	52
Methanol	0.16	0.11	14
n-Butanol	0.49	0.10	14
Octanoic Acid	0.95	0.45	37**
Pharmaceutical	--	0.52	12
Phenol	1.02	0.40	37**
Propionic Acid	0.56	0.37	37**

TABLE (3-2)

(Cont)

Substrate	Reported Yields mg cells/ mg substrate	 mg cells/ mg substrate COD	References
Pulp and Paper Wastes	--	0.52	12
Pyruvic Acid	0.51	0.61	37**
Ray-Rope Wastes	--	0.36 to 0.41	60
Refinery Wastes	--	0.48	12
Sucrose	0.47	0.46	52
Sucrose	0.41	0.40	62
Sucrose	0.69	0.67	37**
Succinic Acid	0.33	0.23	37**
Spent Sulfite Liquor	--	0.37	12
Sulfite Waste Liquor	--	0.68	60
Synthetic Fiber Wastes	--	0.26	12
Synthetic Sewage	--	0.41 to 58	28
Synthetic Sewage	--	0.46	63

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*The circumstances of growth were not usually well defined.
**Computed from Warburg data (62).

A different approach to the problem was recently developed by Bauchop and Elsden(2) who related observed cell growth to the calculated theoretical production of ATP from the energy source during metabolism. Experimental results using anaerobic cultures gave consistent growth yields of 10 µg to 11 µg of cells (dry weight) per µ mole of ATP theoretically formed.

In comparing the data of Bauchop and Elsden with other workers, Gunsalus and Shuster(19) conclude that a value of 10.5 µg of cells per µ mole of ATP formed for anaerobic growth is supported by present experimental knowledge. They also indicated in a calculation of the energy required (Table 3-3) for polymer synthesis in cells, that each µ mole of ATP has the potentiality of forming about 33 µg of cellular substance. The difference between this value and the observed cell yield (10 µg per µ mole of ATP) indicates that polymer synthesis accounts for only 30 percent of the actual energy expanded. They hypothesized that the other 70 percent serves the roles of monomer formation and movement including mechanical motion of flagellar origin, transport of metabolites across membrane and other boundaries, deformation in cell growth, etc.

In reviewing of the relationship between energy and yield for aerobic systems, Gunsalus and Shuster(19) state that imperfect knowledge of reaction steps and of energy coupling (metabolic pathways) in aerobic bacteria restrict the usefulness of available data for thermodynamic purposes. This is in contrast to the more detailed knowledge of anaerobic fermentation mechanisms which per-

TABLE (3-3)*

Energy Expenditure for Polymer Synthesis[a]

Substance	Dry Weight (%)	Monomer Units		ATP Equivalents for Polymerization	
		Average mol. wt.	μ Moles per 100 mg cells	Per Monomer[b]	μ Moles per 100 mg cells
Protein	60	110	545.0	3	1,635
Nucleic Acid	20	300	66.6	5	333
Lipid[c]	10	60	303.0	3	909
Polysaccharide	10	166	60.0	2	120
					2,997

---

*From Gunsalus and Shuster(19)

[a] One hundred milligrams dry weight of cells.

[b] Generalized values.

[c] Calculated as fatty acid.

mits a more quantitative consideration of substrate, products and energy linkage.

The work of engineers up to June 1963 was summarized by Servizi and Bogan(62). They conclude that in the sanitary engineering literature there are two schools of thought regarding the effective yield coefficient. One view is that the quantity of cells produced during biological treatment varies with the chemical nature of the waste. This view is supported by the data of Placak and Ruchhoft(52), Sawyer(60), and McCabe and Eckenfelder(34). The alternate view is that the relationship between synthesis and the nature of the compound is fixed and independent of the nature of the organic matter being assimilated. This view is shared and supported by the data of Helmers, et al.(21), Heukelekian, et al.(23), Hoover, et al.(24) and McKinney(39).

In attempting to develop a rational basis for understanding the conversion of substrate to cell tissue, Servizi and Bogan used the hypothesis originally suggested by Bauchop and Elsden(2) that cell synthesis should be dependent on the yield of ATP. They then postulated that ATP yield is dependent on the change in the free energy of oxidation of the substrate which in turn is directly proportional to the theoretical chemical oxygen demand. They also reported that experimental evidences suggest that the effect of the substrate structure is general rather than specific. Carbohydrates, glycolysis products, and Krebs cycle intermediates appear to lie in one category and aromatics and aliphatic acids in another category.

In a subsequent discussion of this article, McCarty(35) points out that as long as the data of a single investigation was used, the relationship between free energy and cell synthesis appeared to be relatively constant but when data from different investigations are used, there is a significant spread in values for the yield coefficient and noted that this difference is probably due to the difference in the environmental conditions each investigator imposed on the growth of the organisms being studied.

In a second discussion McKinney(35) states that the difference in effective cell yield coefficients is due to endogenous respiration which continuously utilizes the cellular protoplasm formed so that the protoplasm measured will always be less than the protoplasm formed.

In noting the discrepancies in Servizi and Bogan's work these discussions fail to note the error in the basic assumption that ATP yield is dependent on the change in the free energy of oxidation of the substrate. This assumption can easily be shown to be wrong by the following example given by Bauchop and Elsden(2).

Both S. cerevisiae and P. lindneri convert glucose anaerobically to ethanol and CO_2 along with some minor end products. The yeast ferments glucose by the Embden-Meyerhof pathway (Figure 3-1) with an ATP yield of two, whereas P. lindneri catabolized glucose via 2 oxo - 3 deoxy - 6 phosophogluconate (the Entner-Doudoroff pathway) with an ATP yield of one. Since the overall reaction is the same in both cases, the free energy change will also be identical, regardless of the fact that the mechanisms and ATP yields involved differ markedly from one another.

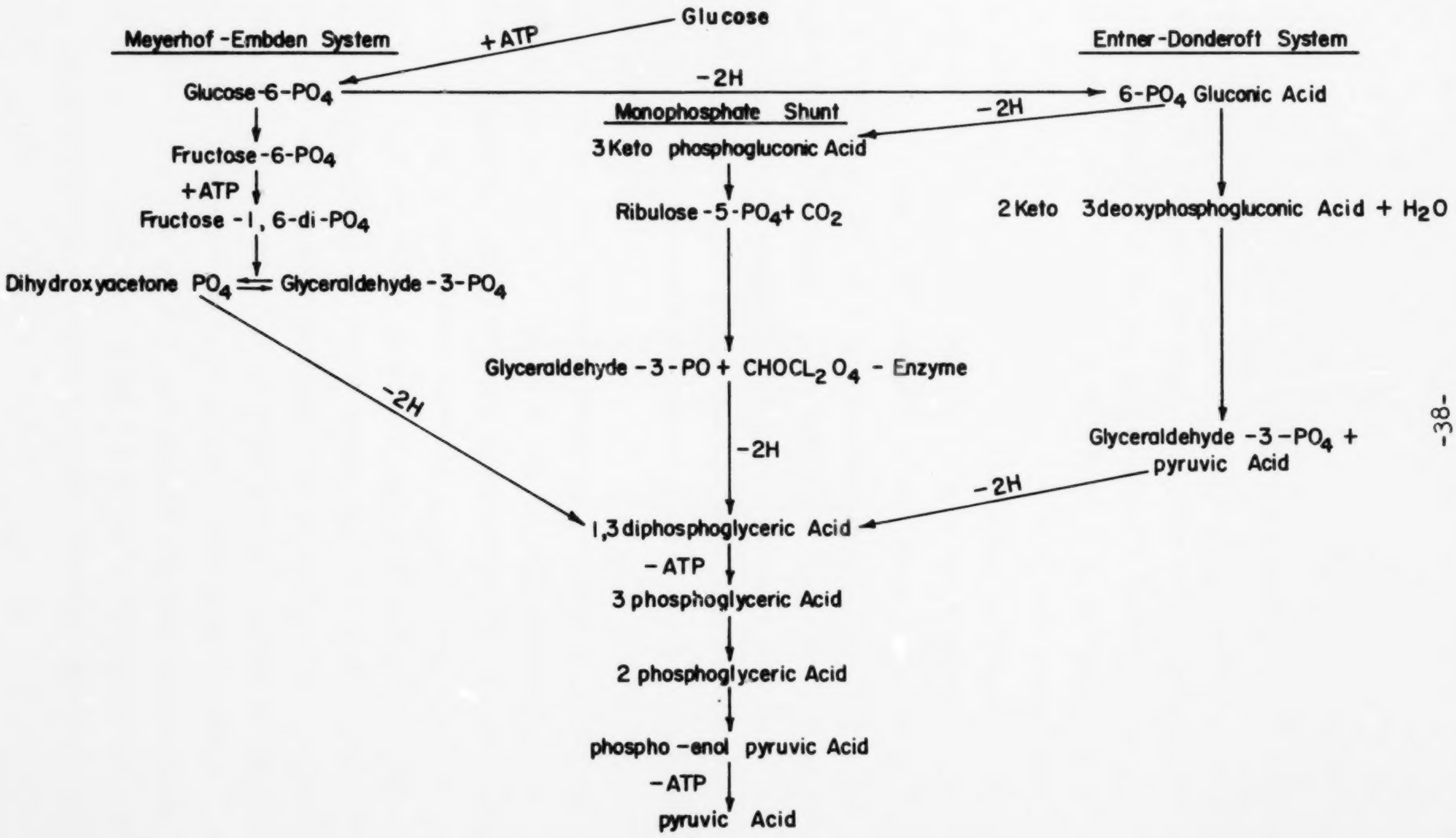

Meyerhof-Embden System
Glucose
+ATP
Entner-Donderoft System
Glucose-6-PO4
-2H
6-PO4 Gluconic Acid
Monophosphate Shunt
-2H
Fructose-6-PO4
3 Keto phosphogluconic Acid
+ATP
Fructose-1, 6-di-PO4
Ribulose-5-PO4+ CO2
2 Keto 3deoxyphosphogluconic Acid + H2O
Dihydroxyacetone PO4
Glyceraldehyde-3-PO4
Glyceraldehyde-3-PO + CHOCL2 O4 - Enzyme
-2H
-2H
Glyceraldehyde-3-PO4 +
pyruvic Acid
-2H
1,3 diphosphoglyceric Acid
-ATP
3 phosphoglyceric Acid
2 phosphoglyceric Acid
phospho-enol pyruvic Acid
-ATP
pyruvic Acid

FIGURE 3-1 METABOLIC PATHWAYS
GLUCOSE TO PYRUVIC ACID

Experimental results of Bauchop and Elsden showing that the yield of S. cerevisiae is double that of P. lindneri support their hypothesis that ATP yield and not free energy is the index of the biological useful energy available to the organisms. Since this first assumption of Servizi and Bogan was wrong, it invalidates their final conclusion.

The concept of alternate or multiple pathways of carbohydrate degradation by the same organism has been amply substantiated. Three possible pathways by which glucose can be catabolized to pruvic acid, a key intermediate of carbohydrate metabolism, are shown in Figure (3-1). There are other possible paths but the three given here have all been shown to be utilized by P. fluorescens(76). It can be seen that for aerobic systems (assuming a phosphorylation quotient of DPNH equal to 3) there is an ATP yield of 8 if the Meyerhof-Embden System is utilized, of 7 if the Entner-Donderoft System is used and 10 for the Monophosphate Shunt.

The theoretical calculation of ATP yield and energy linkages in this situation becomes extremely difficult. An organism may possess primarily a Meyerhof-Embden System but an alteration in growth conditions may permit other paths to predominate.

EXPERIMENTAL PROCEDURES

Materials

A homogeneous reactor of the type described above and similar to the continuous culture apparatus developed and used by microbiologists for the study of bacteria(22)(26)(41)(42)(52)(57) was constructed.

The continuous culture system in its simplest form (Figure 3-2) consists of a reaction vessel to which medium with a fixed substrate concentration, S_1, is added at a constant flow rate, Q. The volume, V, of the reaction vessel is kept constant by an overflow. The fluid in the vessel is kept completely mixed so that its contents can be considered homogeneous. The temperature, pH, pressure, and other environmental conditions were considered to be constant. Therefore, they were not considered in the mathematical development given above. The effects of the toxic end products of metabolism were considered to be minor for the substrate concentration being considered and were neglected. A schematic sketch of the entire continuous culture apparatus is given in Figure 3-3.

The apparatus was operated using pure cultures of bacteria of the following species:

1. *Pseudomonas fluorescens*
2. *Escherichia coli B*
3. *Alcoligenes facalis B*
4. *Bacillus cereus*

Pure cultures of these organisms were obtained from Dr. H. Ehrlich of the Rensselaer Polytechnic Institute, Department of Microbiology, and were maintained on nutrient agar slants stored at 4°C. These organisms were selected because the literature indicated they are typical of an activated sludge community. Bacteria are the predominant organisms in activated sludge and it is assumed that the overall physiological properties of the mixed cultures found in activated sludge systems will, in general, be similar to the properties of pure cultures of bacteria.

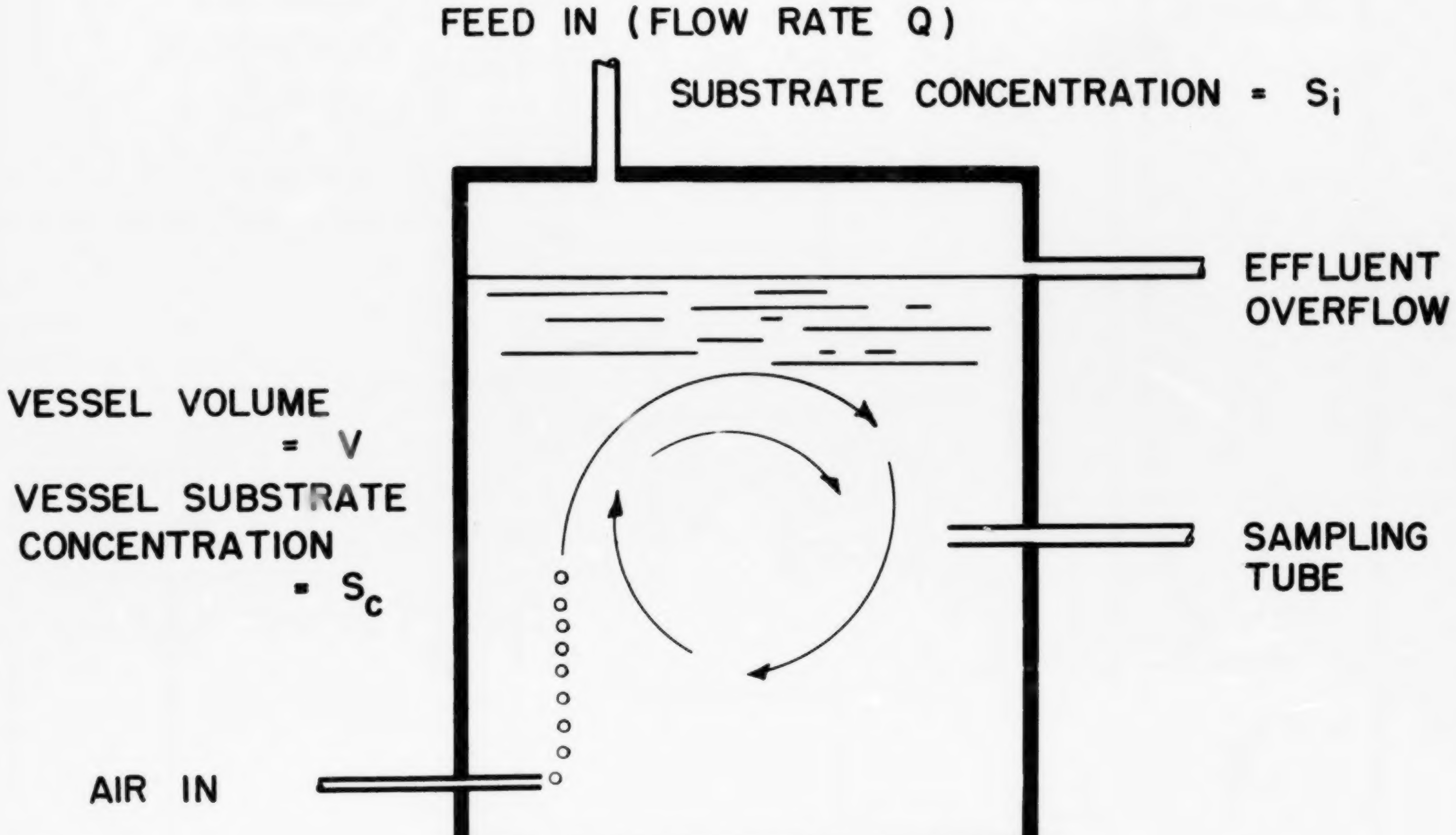

FEED IN (FLOW RATE Q)
SUBSTRATE CONCENTRATION = S_i
EFFLUENT OVERFLOW
VESSEL VOLUME = V
VESSEL SUBSTRATE CONCENTRATION = S_c
SAMPLING TUBE
AIR IN

FIG. 3-2

CONTINUOUS CULTURE REACTION VESSEL

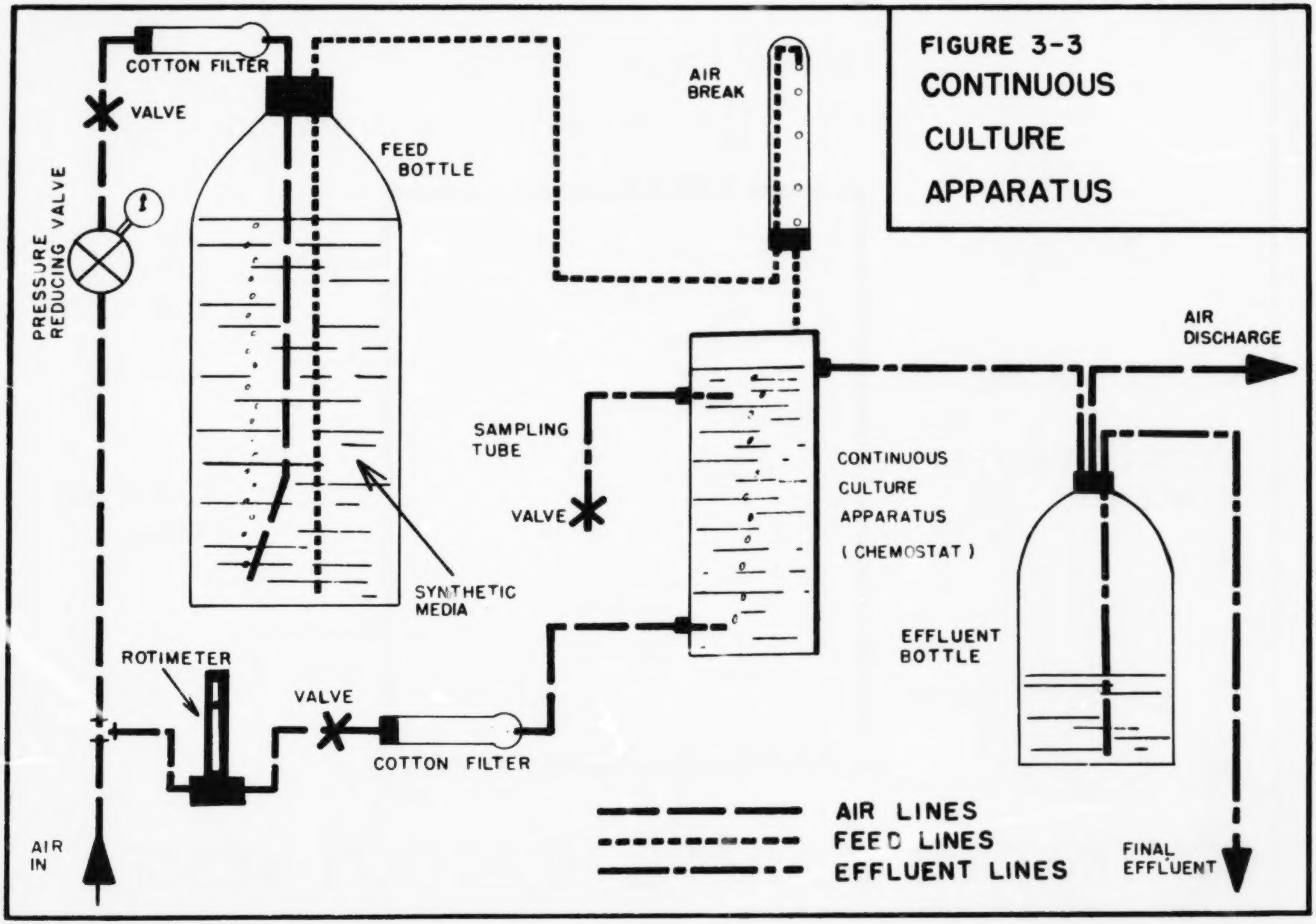


FIGURE 3-3
CONTINUOUS
CULTURE
APPARATUS

COTTON FILTER
VALVE
PRESSURE REDUCING VALVE
FEED BOTTLE
SYNTHETIC MEDIA
AIR BREAK
SAMPLING TUBE
VALVE
CONTINUOUS CULTURE APPARATUS (CHEMOSTAT)
AIR DISCHARGE
EFFLUENT BOTTLE
ROTIMETER
VALVE
COTTON FILTER
AIR IN
AIR LINES
FEED LINES
EFFLUENT LINES
FINAL EFFLUENT

Glucose, acetate and glutamic acid were chosen as substrates because of the simplicity of their organic structure and since they represent the major components of sewage, i.e. carbohydrates, fatty acids and amino acids. A synthetic sewage, (after McKinney(38)) and an industrial waste (see Appendix Table B-1) were also used in order to compare the results with other workers Appropriate quantities of buffer (pH = 7.0) and metallic trace elements were added to all of the media and they were made up to a final COD concentration of 1000 mg/l.

Analyses

Samples were analyzed for total COD, dissolved COD, total count, pH and by gram stains.

The COD was measured by a modification of the method described in Standard Methods.(1) The modified method used one-fifth of the suggested volume of the sample and reagents. The silver sulfate catalyst was used in all the determinations. The dissolved COD was found by measuring the sample after it was filtered through a membrane filter (Gelman #GM-6, Pore Size = 0.45). A study of the precision of the test using acetic acid demonstrated a standard deviation of 19 mg/l using a sample with a mean COD = 363 mg/l. This is equivalent to a coefficient of variation of 5.2%. The mean COD was 93.7% of the theoretical oxygen demand.

The total count of bacteria was measured using a Petroff-Hausser bacteria counting chamber and a phase contrast microscope. A study of the precision of this test demonstrated a standard deviation of 1.9×10^9 using a sample containing 3.1×10^{10} bacteria per ml. This is equivalent to a coefficient of variation of 6.1%.

The pH was measured by a Coleman Model 31 pH meter.

The gram stains were made according to the modification of Huckler given in Standard Methods(1).

OPERATING PROCEDURES

Before each run the entire apparatus and the media were sterilized by autoclaving. The reaction vessel was then filled with media and inoculated with a suspension of the desired organisms made by washing an agar slant. Growth was allowed to proceed until the concentration of organisms in the reactor was a maximum. Medium flow through the culture vessel was then started at the desired flow rate. When steady-state conditions were reached in the reactor (experience indicated that this requires between 5 and 10 hours) a sample was taken. The medium flow rate was then changed and a new steady state examined.

An example of steady state conditions is shown by Figure 3-4 which shows the results of measurements of bacterial densities and flow rates that were taken periodically for 36 hours. Although fluctuations did occur, they were small and were within the errors in measurements.

The purity of the culture in the reaction vessel was periodically checked by gram stains. When there was any cause to doubt the purity of the culture, the apparatus was taken down, resterilized, and reinoculated from the stock culture.

RESULTS

The following runs using different combinations of organisms and substrates were made.

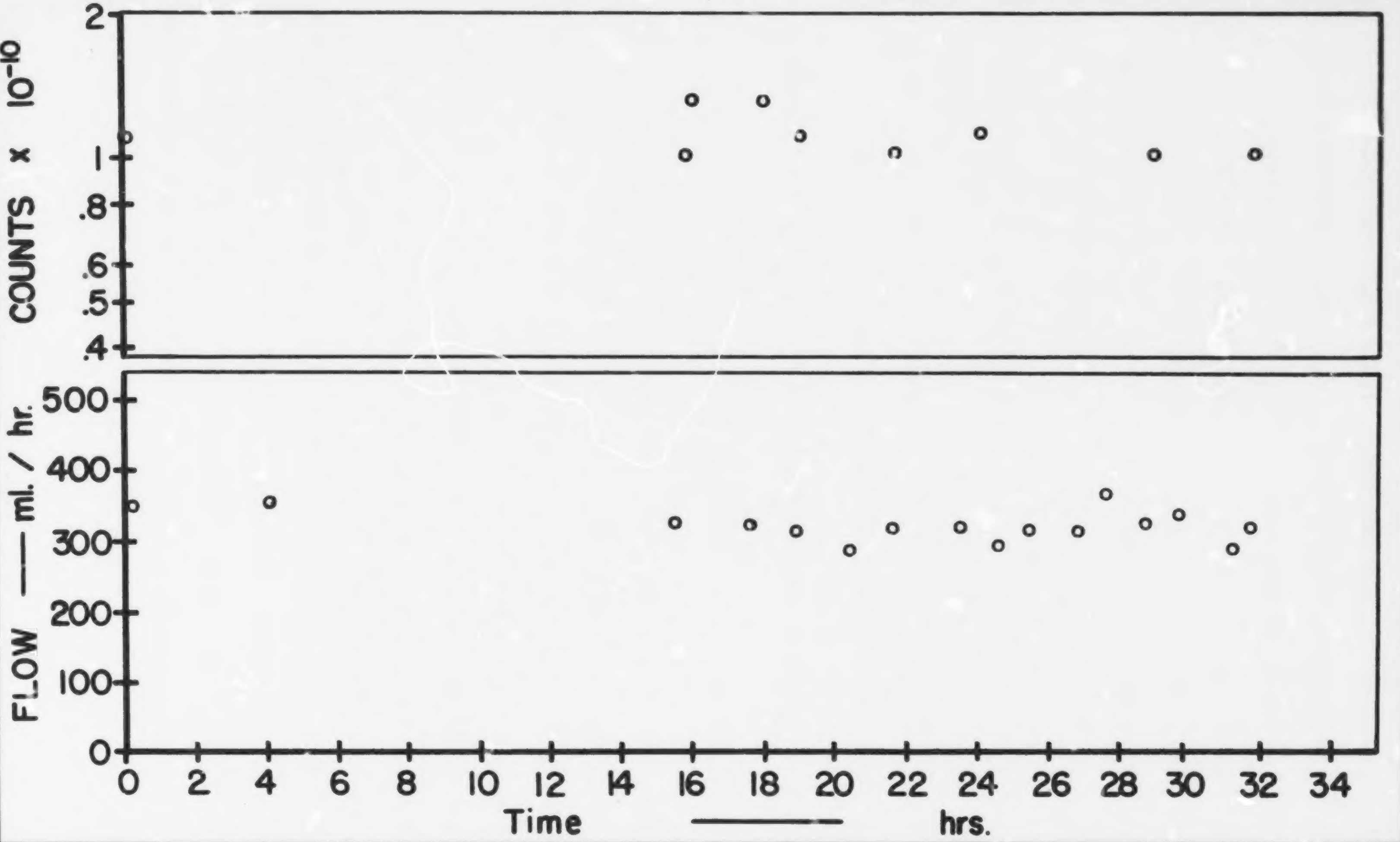


FIGURE 3-4—— STEADY STATE CONDITIONS IN THE REACTOR

CONTINUOUS CULTURE RUN 2

PSEUDOMONAS FLUORESCENS UTILIZING GLUTAMIC ACID

Run #1	Pseudomonas fluorescens	utilizing	Glucose
Run #2	Pseudomonas fluorescens	utilizing	Glutamic Acid
Run #3	Pseudomonas fluorescens	utilizing	Acetate
Run #4	Escherichia coli B	utilizing	Acetate
Run #5	Escherichia coli B	utilizing	Glutamic Acid
Run #6	Alcaligenes faecalis	utilizing	Glutamic Acid
Run #7	Bacillus cereus	utilizing	Glutamic Acid
Run #8	Pseudomonas fluorescens and Alcaligenes	utilizing	Glutamic Acid
Run #9	Pseudomonas fluorescens, Alcaligenes faecalis and Bacillus cereus	utilizing	Glutamic Acid
Run #10	Pseudomonas fluorescens	utilizing	A mixture of monomers
Run #11	Pseudomonas fluorescens	utilizing	An industrial waste
Run #12	Pseudomonas fluorescens	utilizing	Synthetic Sewage

For the first five runs, the entire range of dilution rates was covered. For the remaining combinations only the low flow dilution rates were covered. The analytical results of these experiments are shown in Appendix Tables B-2 through B-7.

In order to minimize the inherent inaccuracies of the COD measurements and the slight random fluctuations observed in the cultures at a steady state, the COD data was grouped(6) by dilution rate intervals. The mean of the total and dissolved COD was found for each interval. The cell COD for each interval was then calculated by subtracting the mean dissolved COD from the mean total COD for the interval. The yield coefficient was calculated as:

$$Y = 0.68 \left(\frac{\text{Total COD - Dissolved COD}}{\text{Feed COD - Dissolved COD}}\right)$$

where 0.68 is the theoretical ratio of cell weight to cell COD found by using the generally accepted emperical chemical formulation of $C_5H_8O_2N$ for cells$^{(12)}$. The results of these computations are shown in Appendix Table B-8 for the first five runs.

Heterogenous Metabolism Rate

In order to check the validity of Equation (3-13) plots of $1/Y$ vs T_d were made for the various combinations studied and for the data of Rosenberger and Kogut$^{(57)}$. These plots are shown in Figures (3-5) through (3-10). The resulting straight lines confirmed the assumption made in the development of Equation (3-12). The intercept and slope of the line of best fit was calculated by the method of least squares and the values of $(K_1 + K_2/C)$ and C were determined. The value of C and $(K_1 + K_2/C)$ calculated for the organism and substrate used are shown in Table (3-4).

These data indicate that the values of $(K_1 + K_2/C)$ are not constant for all organisms and substrates. The combinations measured gave a range of values for metabolism coefficient, $(K_1 + K_2/C)$, from 0.010 to 0.067 mg substrate COD/mg cell/hr. Unfortunately, these values could not be further subdivided to calculate the values of K_1 and K_2. If the specific substrate had been separated from the total substrate, it may have been possible to evaluate K_1 independent of the above expression. Then by using the values of the metabolism coefficient given in Table (3-4) an estimate of K_2 could have been made. The value of such an approach is, however, questionable. The data used in plotting Equation (3-13) was

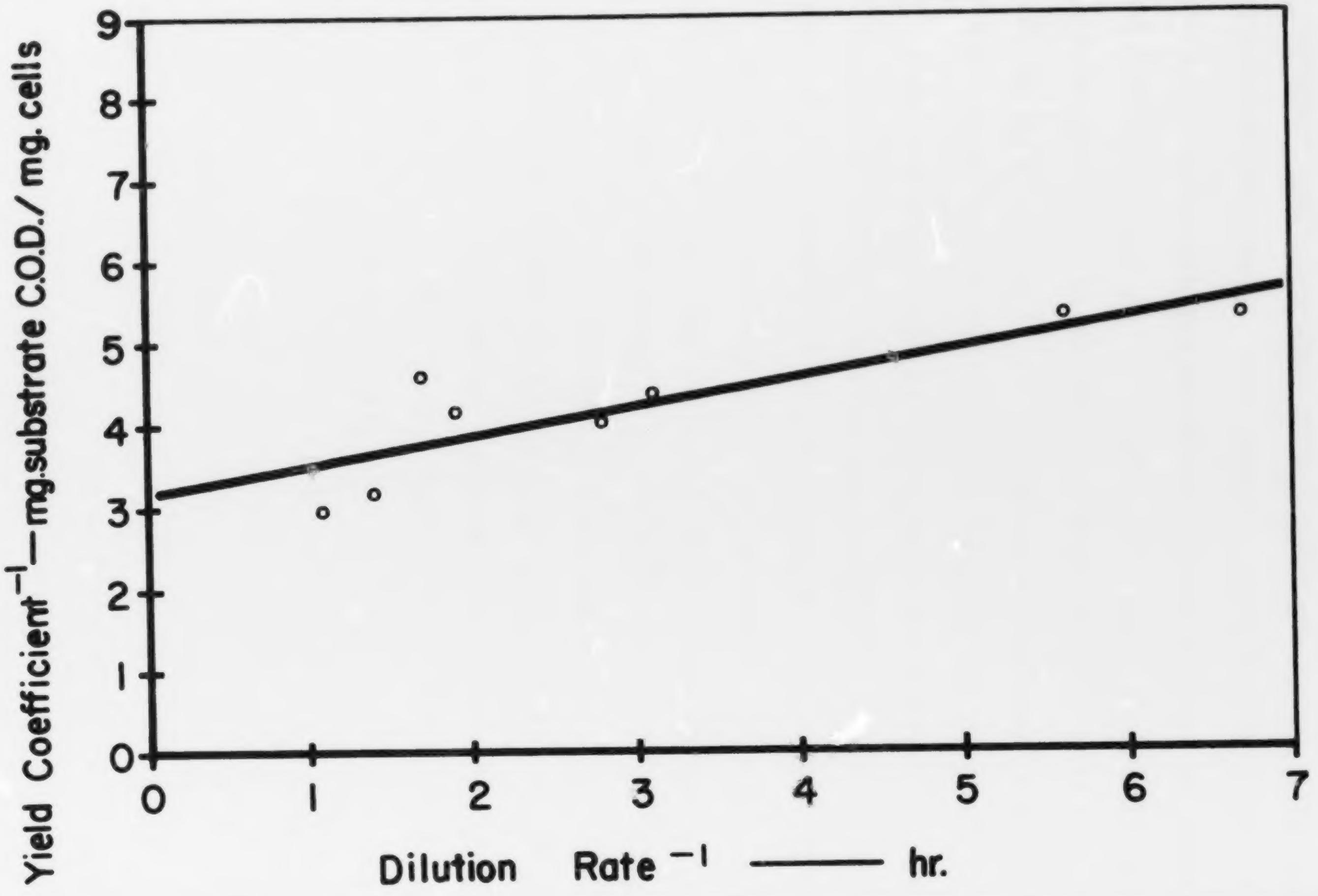


FIGURE 3-5 —— YIELD COEFFICIENTS

CONTINUOUS CULTURE RUN I

PSEUDOMONAS FLUORESCENS UTILIZING GLUCOSE

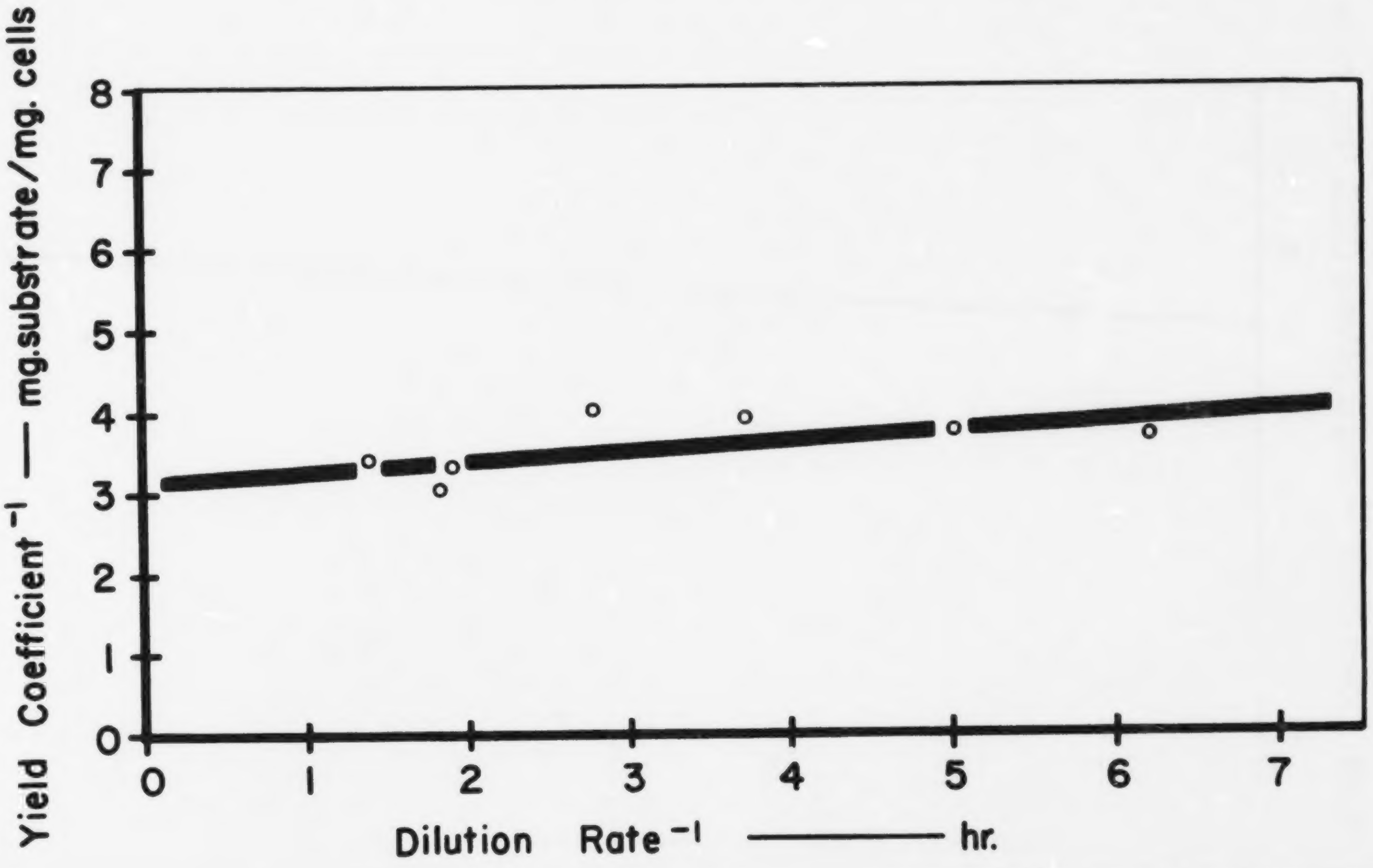


FIGURE 3-6 —— YIELD COEFFICIENTS
CONTINUOUS CULTURE RUN 2
PSEUDOMONAS FLUORESCENS UTILIZING GLUTAMIC ACID

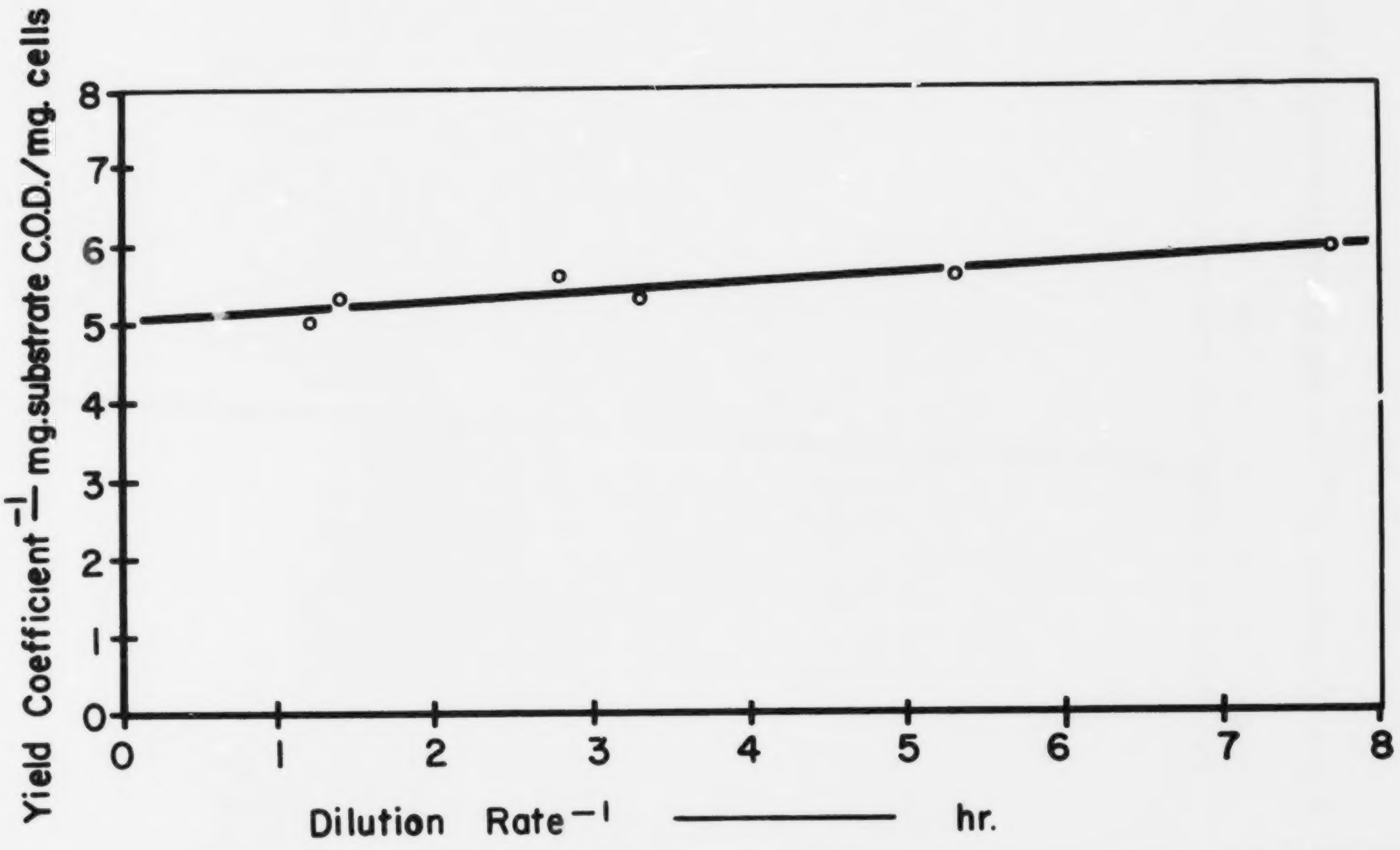


FIGURE 3-7 —— YIELD COEFFICIENTS
CONTINUOUS CULTURE RUN 3
PSEUDOMONAS FLUORESCENS UTILIZING SODIUM ACETATE

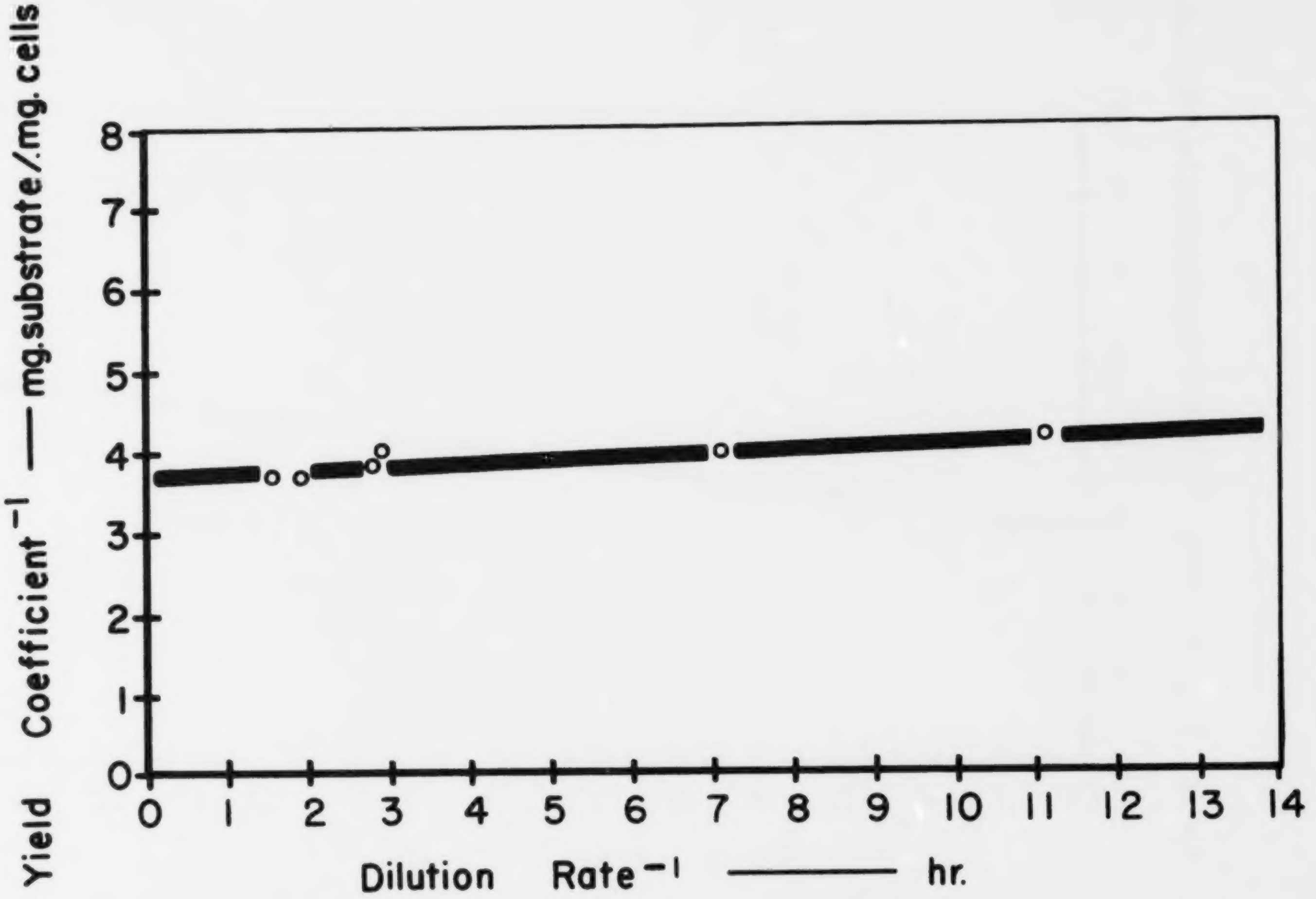


FIGURE 3-8 —— YIELD COEFFICIENTS
CONTINUOUS CULTURE RUN 4
ESCHERICHIA COLI B UTILIZING SODIUM ACETATE

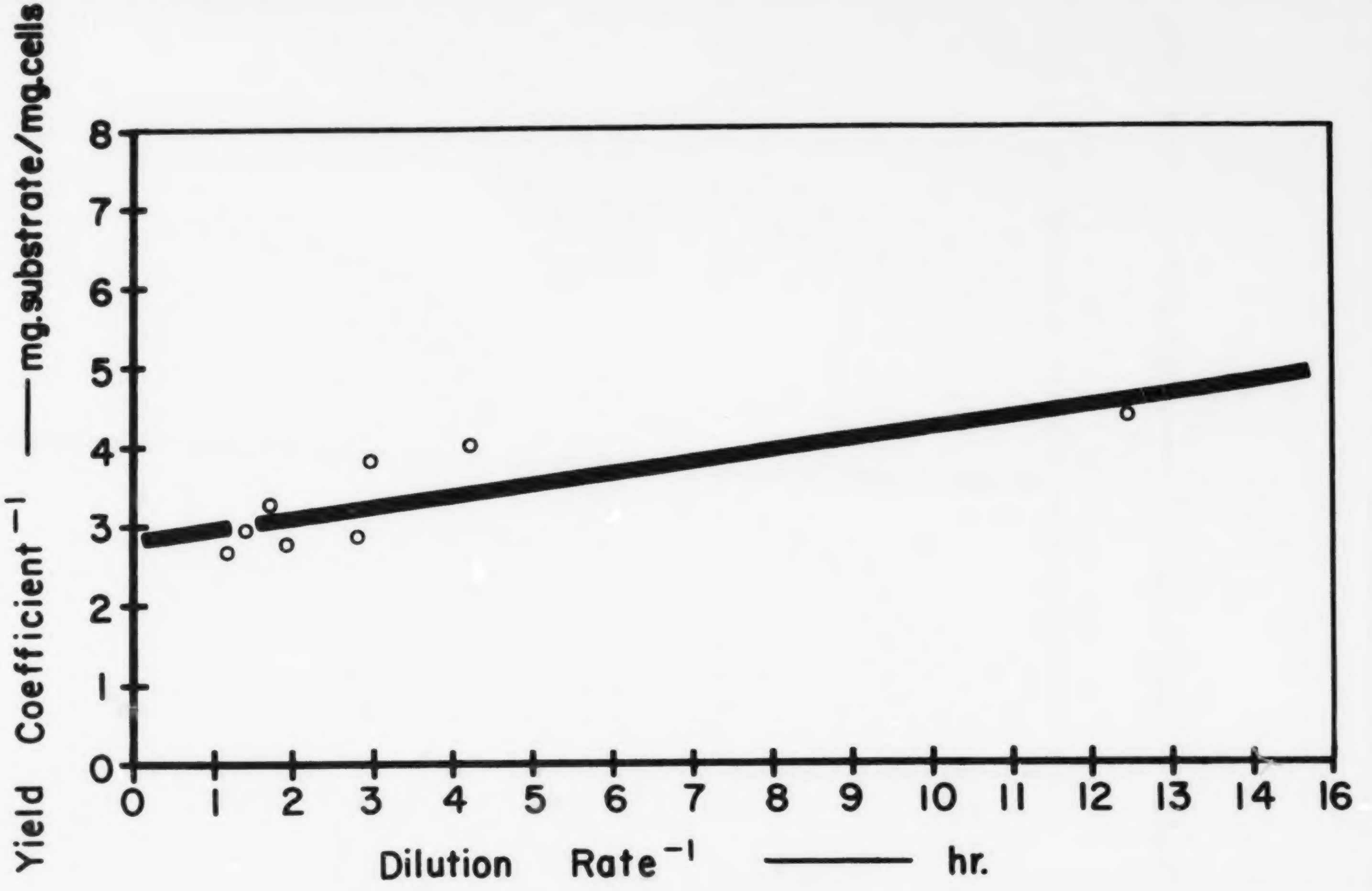


FIGURE 3-9 —— YIELD COEFFICIENTS
CONTINUOUS CULTURE RUN 5
ESCHERICHIA COLI B UTILIZING GLUTAMIC ACID

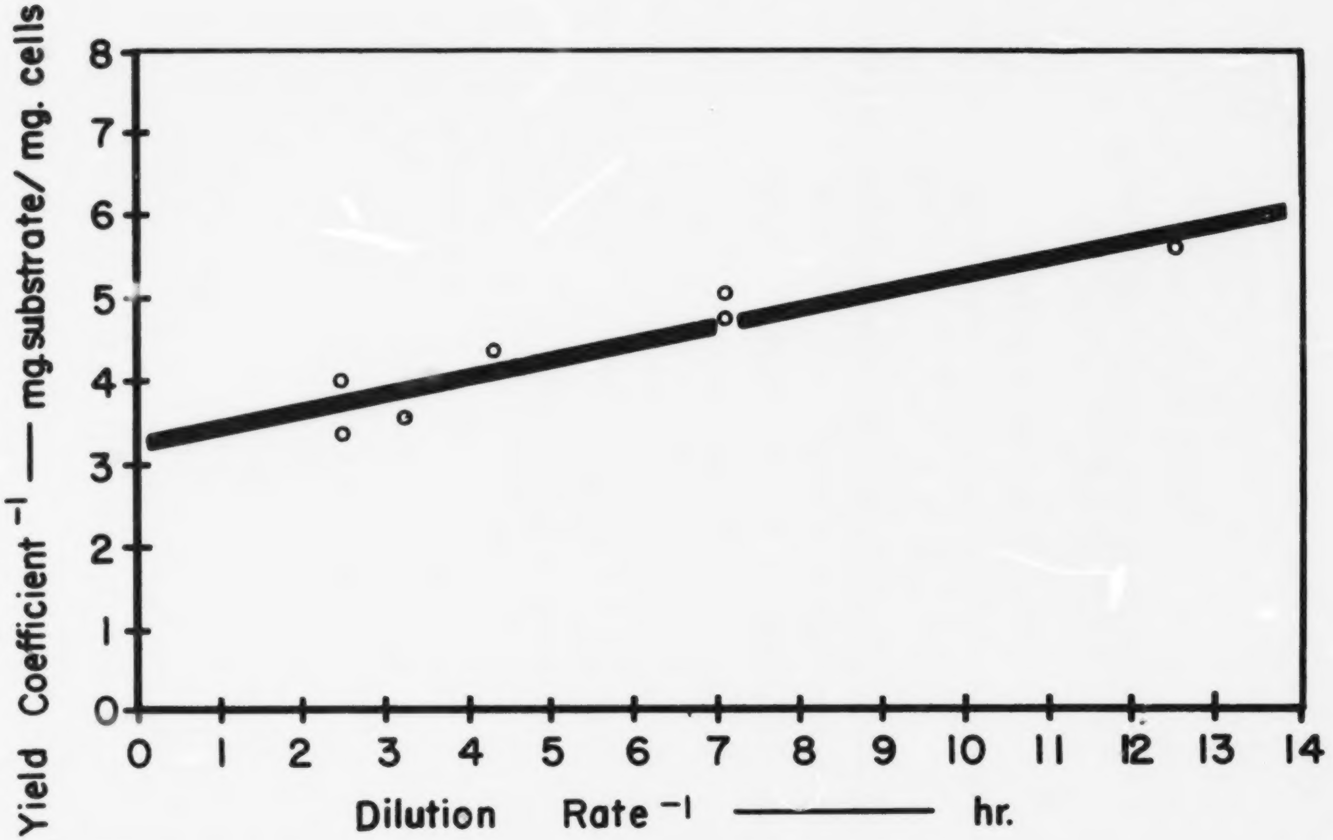


FIGURE 3-10 —— YIELD COEFFICIENTS
CONTINUOUS CULTURES (DATA FROM ROSENBERGER & KOUGT.)
PSEUDOMONAS FLUORESCENS UTILIZING SUCCINATE

TABLE (3-4)

Calculated True Yield Coefficients and Energy Metabolism Rates

Summary of Results

Organism	Substrate	True Yield Coefficient	Metabolism Coefficient
Pseudomonas fluorescens	Glucose	0.31	0.067
Pseudomonas fluorescens	Glutamic Acid	0.35	0.015
Pseudomonas fluorescens	Sodium Acetate	0.19	0.031
Escherichia coli B	Sodium Acetate	0.27	0.010
Escherichia coli B	Glutamic Acid	0.35	0.021
Pseudomonas fluorescens	Succinate	0.19	0.039

obtained for several dilution rates. However, the calculation procedure uses, as a primary point, the value of 1/Y at a zero dilution rate. There is some doubt that the equation can be extrapolated outside of the range of the available data. The questionable nature of this approach is emphasized by the transfer rates considered in Chapter V.

The data also show that the values of the calculated true yield coefficients (C) vary from 0.19 to 0.35. This indicates that even if the effective yield coefficient (Y) is corrected for heterogenous metabolism the quantity of cells synthesized for each unit of substrate uitlized is not constant but varies with substrate and organism.

Variations in the Effective Yield Coefficient with Substrate and Organism

In comparing the effective yield coefficients found for the different combinations of substrates and organisms used, (see Table 3-5) the following was observed:

1. The effective yield coefficients are not constant with substrate and organism but vary from 0.16 to 0.35.
2. *E. coli B* gives a higher yield coefficient than *P. fluorescens* when growing on acetate.
3. *P. fluorescens*, *B. cereus*, and *A. Faecalis* give a higher yield coefficient than *E. coli B* when growing on glutamic acid.

TABLE 3-5

Variations in the Effective Yield Coefficient with Organism and Substrate

Summary of Results

Organism	Substrate	Effective Yield Coefficient mg cells/ mg Substrate COD	Dilution Rate
Alicaligenes faecalis	Glutamic Acid	0.27	0.12
Bacillus cereus	Glutamic Acid	0.27	0.18
P. fluorescens and A. faecalis	Glutamic Acid	0.29	0.17
P. fluorescens, A. faecalis and B. cereus	Glutamic Acid	0.31	0.17
P. fluorescens	Mixed Monomers	0.29	0.13
P. fluorescens	Industrial Wastes	0.35	0.13
P. fluorescens	Synthetic Sewage	0.29	0.14
P. fluorescens	Glucose	0.17	0.15
E. coli B	Sodium Acetate	0.24	0.15
E. coli B	Glutamic Acid	0.28	0.15
P. fluorescens	Glutamic Acid	0.27	0.15
P. fluorescens	Sodium Acetate	0.16	0.15

4. The yield of B. cereus, A. faecalis, and P. fluorescens on glutamic acid is higher when grown together as a mixed culture than when grown as a pure culture.
5. The yields of P. fluorescens are higher when utilizing the complex media (synthetic sewage, the industrial waste or the mixture of the three monomers) than when utilizing the simple media made of a single monomer, (glucose, glutamic acid or acetate).

DISCUSSION

The above noted variations in the effective yield coefficient can be explained in part by the effect of the die off and energy metabolism and in part by the theory relating yield to ATP formation.

The yield of ATP from any substrate depends not only on its free energy, but also its metabolic pathway. Therefore, an organism may degrade the same substrate and get different yields because it uses different metabolic pathways which give different yields of ATP. Since the system must be thermodynamically balanced, these variations in yield suggest that there must be other mechanisms of energy dissimilation other than formation of new protoplasm. Gunsalus and Shuster(19) suggest the following three possibilities:

1. Accumulation of high energy polymeric produces, either in storage form or as usable waste.

2. Dissipation as heat by ATP.
3. Activation of shunt mechanisms bypassing energy yielding reactions or requiring a greater expenditure of energy for priming.

The use of substrate to provide energy for these purposes would cause an increase in the heterogenous metabolism rate.

If the quantity of cells synthesized is proportional to the ATP yield, synthesis must also be dependent not only on the ΔF but also on the metabolic pathways by which the compound is degraded. Servizi and Bogan have shown that COD is directly proportional to the free energy of a compound thus the yield coefficient, when measured in terms of mg cells/mg substrate COD utilized, will also vary if the substrate is degraded by different metabolic pathways.

The smaller yields found for single substrates can be attributed to energy required for monomer synthetics. Since many different monomers are present in the complex media, they need not be synthesized by the cells and the energy normally used for synthetics of monomers can be used for polymer synthetics and will result in higher cell yields.

The effect of mixed cultures is somewhat more complicated. The question of the relationship of the consistency between energy liberating reactions and cell growth must first be raised. What happens when cell growth is not limited by lack of an energy source but by some other factor such as unfavorable chemicals or physical environment or by the lack or scarcity of a required growth factor? This problem has been studied to a limited extent by McIlwain[19]. He concludes that in the case of a limiting factor other than an energy source, the substrate may continue to be degraded either at full or reduced rates even though cell growth has ceased. In these cases, the available energy cannot be used for growth and must be dissipated in some other fashion. Mechanisms for dissipating excess energy have already been discussed.

In light of the above, the lower yield observed for pure cultures can be explained if we assume that their rate of growth of a pure culture growing on a simple media is being slowed down by the absence of some other factor than the energy source (in this case most likely an accessory growth factor). The increase in growth when several organisms are grown together is explained by the phenomenon of syntrophism; i.e. the accessory growth factor required by organism A is synthesized in excess by organism B and released to the surrounding media. Organism A's growth is thus able to proceed at a more rapid rate, (from the organism point of view), a more efficient use of energy is possible.

This lack of accessory growth factors can also be used to explain the difference in yields when the simple monomers are used as a substrate and when a complex media such as the synthetic sewage is used. The synthetic sewage which contains these accessory growth factors gives a greater yield than the monomers that do not contain them.

SUMMARY

The data and theory discussed above indicate that the effective yield coefficient (Y) will vary with different substrates and organisms or even with the same substrate and organism depending on the metabolic pathway by which the substrate is degraded. The absence of a key enzyme or a limiting amount of one enzyme for substrate turnover to serve in the cellular energy supply system of an organism may shunt substrate to an alternate pathway less favorable energetically for biosynthesis. The significant variations in the effective yield with dilution rate coefficient may be the result of heterogenous metabolism of the organisms.

The true yield coefficient is a function of the ATP production but it is not necessarily proportional to the free energy of oxidation of the substrate utilized. The true yield coefficient was, in fact, observed to vary for the same substrate when metabolized by different organisms. Mixed cultures gave higher yields than did pure cultures. Also, the complex medium studied gave a higher yield than did the simple media.

CHAPTER IV - STEADY STATE BACTERIAL CULTURE RATE OF SYNTHESIS

INTRODUCTION

Chapter III dealt with the determination of the amount of volatile solids which will be produced by bacterial systems at any given process loading as measured by chemical techniques widely used in the field of Environmental Engineering. In this chapter the same techniques were used to determine the changes in the rate of synthesis at various process loadings. In the continuous culture apparatus used for these studies, the process loading was controlled by the dilution rate. The process loading, in turn, controlled the rate of synthesis of the culture.

One of the more important relationships in the design of a biochemical waste treatment process is the relationship between the rate of growth of the microorganism and the substrate concentration in the system. This fundamental relationship must be understood both qualitatively and quantitatively for proper design and operation of these systems. A review of the literature reveals some confusion and disagreement on this relationship by both biologists and engineers. Of the biologists, Monod, in attempting to clarify this situation, first studied it using batch culture. He concluded that the effective growth rate of viable organisms, μ_e, at a particular substrate concentration, S_c, is equal to the maximum growth rate, μ_m, times the ratio of the substrate concentration, S_c, to sum of S_c and the substrate concentration that gives one half the maximum growth rate, K_s.

$$\mu_e = \mu_m \left(\frac{S_c}{S_c + K_s}\right) \qquad (4\text{-}1)$$

This is also the form of the generally accepted Michaelis-Menten relationship for enzyme substrate interaction(48). Many microbiologists take the point of view that since absorption, transport, and enzymatic utilization of an agent essential to growth fit this general category of reactions, the relationship could be expected to hold for the overall growth(25)(33)(42). Whether or not this theoretical basis is justified has been questioned(43)(54) but the equation fits experimental data rather well.

Other relationships have been proposed by biologists mainly because they give a better fit of the data available. The relationship

$$\mu_e = \mu_m \left(1 - e^{-yS_c}\right) \qquad (4\text{-}2)$$

was used by Teissier(70), Spencer(67), and Lipe(29).

$$\mu_e = \mu_m \left(\frac{S_c}{yMa + S_c}\right) \qquad (4\text{-}3)$$

was proposed by Contois(5) and

$$\mu_e = \mu_m \left(\frac{S_c}{y - S_c x}\right) \qquad (4\text{-}4)$$

by Moser(45).

A review of the engineering literature indicated that most engineers assume that when substrate concentration becomes limiting, the rate of increase of microbial mass, usually measured as mixed liquor suspended volatile solids, MLVS, is directly proportional to the gross measure of the substrate concentration (S_c')(12)(27)(39).

$$\mu_e'' = KS_c' \qquad (4\text{-}5)$$

where μ_e'' is the effective growth rate of total volatile mass in the process. Since the MLVS may contain a variable amount of active organism, it is preferable to consider the growth rate of the active mass

$$\mu_e = fS_c' \qquad (4\text{-}5a)$$

Equation (4-5a) is then directly comparable to the equations for μ_e reported in the biological literature.

Schulze(61) studied the continuous growth of E. coli on an organic substrate of glucose and urea. Based upon his analyses of the removal of glucose, he preferred the use of Equation (4-2) to express the relation of growth rate to substrate concentration. (It should be noted here that Lipe(29) worked with Schulze on his research.) He further suggested that this equation would apply to activated sludge cultures.

However, Downing and Wheatland(8), Rich(56), and Steward and Ludwig(68) have proposed the use of Equation (4-1). As mentioned previously, available data tend to support Equation (4-1), but in every case where substrate was measured, it was measured in terms of the substrate. Engineers use COD (Chemical Oxygen Demand) as a basis for measuring substrate concentration. No data were available relating the growth rate to the substrate concentration measured as COD.

APPROACH

The specific purpose of this research was to determine under laboratory conditions the relationship of substrate concentration to growth rate where the substrate concentration is measured as COD.

In order to study the relationship between substrate concentration and growth rate, an open, homogeneous, continuous culture apparatus, as described in Chapter III, was constructed and operated under various growth conditions using different organisms and substrates. As in the previous study, the apparatus was operated using pure cultures of bacteria since preliminary studies have shown that a steady state could not be maintained using a mixed population similar to activated sludge. Here again, it was assumed that the overall physiological properties of the mixed cultures found in activated sludge will, in general, be similar to the properties of pure cultures of bacteria.

As shown previously, for a homogeneous reactor,

$$\frac{dM_a}{dt} = \mu M_a - K_2 M_a - DM_a \qquad (3\text{-}1a)$$

where M_a represents the concentration of active organism, μ is the true growth rate, and K_2 is the heterogenous metabolism coefficient.

The first term on the right side of Equation (3-1a) is the synthesis of protoplasm per unit time occurring from the metabolism of the organic substrate. The second term represents the destruction of the mass per unit time. Neither of these terms are measured directly by researchers. Rather, the effects of both terms are measured together which gives rise to the effective growth rate, μ_e. Most researchers have assumed that the heterogenous metabolism coefficient is insignificant for the conditions of their work. This assumption, justifiable or not, has lead to the development of Equations (4-1) through (4-5a), based upon the effective growth rate. It is readily apparent that K_2 should not influence μ. The magnitude of the error introduced by including the effect of K_2 in growth rate equations and possible corrective measures will, however, not be considered herein.

PROCEDURES

The detailed procedures used in selecting materials, operating the system, and collecting and analyzing samples have been presented in Chapter III. These procedures are summarized below.

A homogeneous reactor of the type described above and similar to the continuous culture apparatus developed and used by many microbiologists for the study of bacteria was constructed.

The apparatus was operated using pure cultures of bacteria of Pseudomonas fluorescens and Escherichia coli B.

Glucose, acetate and glutamic acid were chosen as substrates. Appropriate quantities of buffer (pH 7.0) and metallic trace elements were added to all of the media and they were made up to a final COD concentration of 1000 mg/l.

Before each run the entire apparatus and the media were sterilized by autoclaving. The reaction vessel was then filled with media and inoculated. Growth was allowed to proceed until the concentration of organisms in the reactor was at a steady state. The temperature was maintained at 30°C.

Samples were analyzed for dissolved COD, total count, pH, and by gram stains.

RESULTS

The analytical results of these experiments were given in Appendix Tables (B-2) through (B-7). From the data, plots of dilution rate (D) versus dissolved organics as COD (S_c') were prepared. As shown in Figures (4-1) through (4-5), the data has definite similar trends from all systems with some scattering. The scattering is not surprising upon consideration of the reproducibility of the COD analysis(69). Because of the appearance of these graphs, it was decided to fit to the data a curve for a rectangular hyperbola of the type:

$$D = \mu_m \left(\frac{S_c' - A}{S_c' - B}\right) \qquad (4\text{-}7)$$

It is apparent that Equation (4-7) is equivalent to Equation (4-1) when a gross measure of organic concentration (S_c') is substituted for the specific substrate concentration (S_c). Therefore,

$$A = S_c' - S_c \qquad (4\text{-}9)$$

$$B = K_s - A \qquad (4\text{-}10)$$

The A may be thought of as the concentration of end products and autolysis products in the solution.

Equation (4-7) can be written as:

$$\frac{S_c' - A}{D} = \frac{S_c'}{\mu_m} + \frac{B}{\mu_m} \qquad (4\text{-}10)$$

this is the equation of a straight line of the type

$$y = ax + b \qquad (4\text{-}11)$$

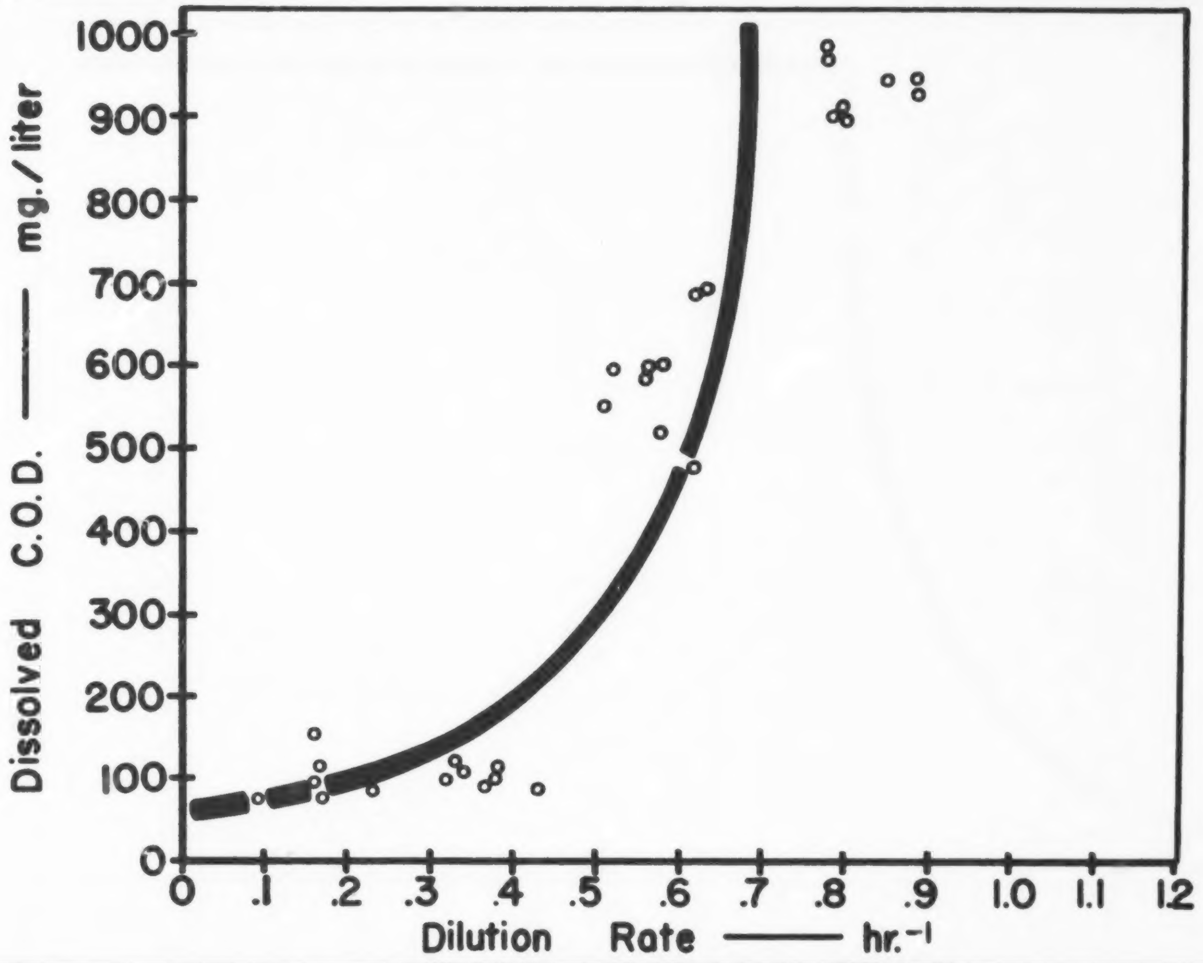


FIGURE 4-1 —— EFFECT OF DILUTION RATE ON DISSOLVED C.O.D.

CONTINUOUS CULTURE RUN I

PSEUDOMONAS FLORESCENS UTILIZING GLUCOSE

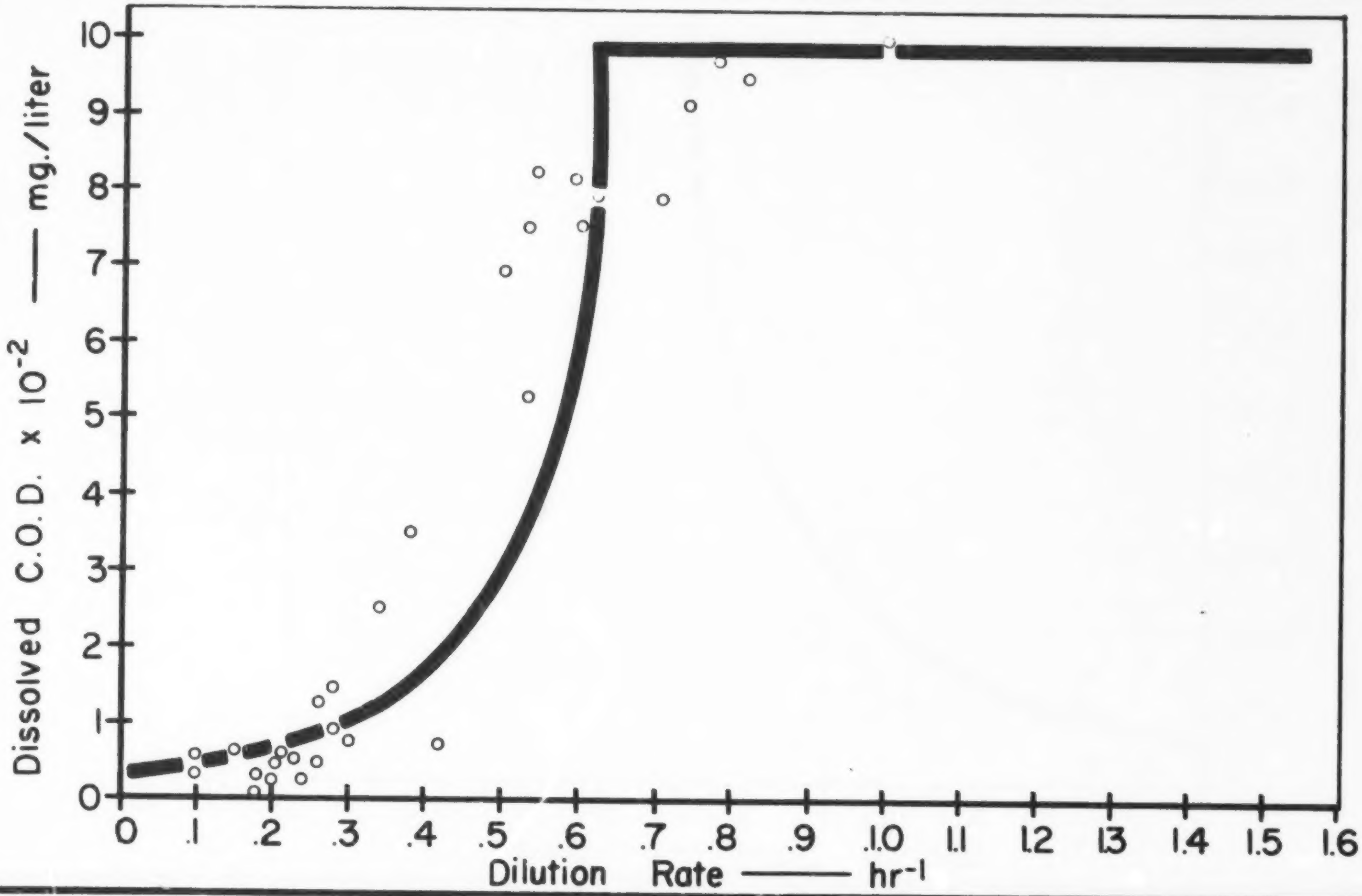


FIGURE 4-2 —— EFFECT OF DILUTION RATE ON DISSOLVED C.O.D.

CONTINUOUS CULTURE RUN 2

PSEUDOMONAS FLUORESCENS UTILIZING GLUTAMIC ACID

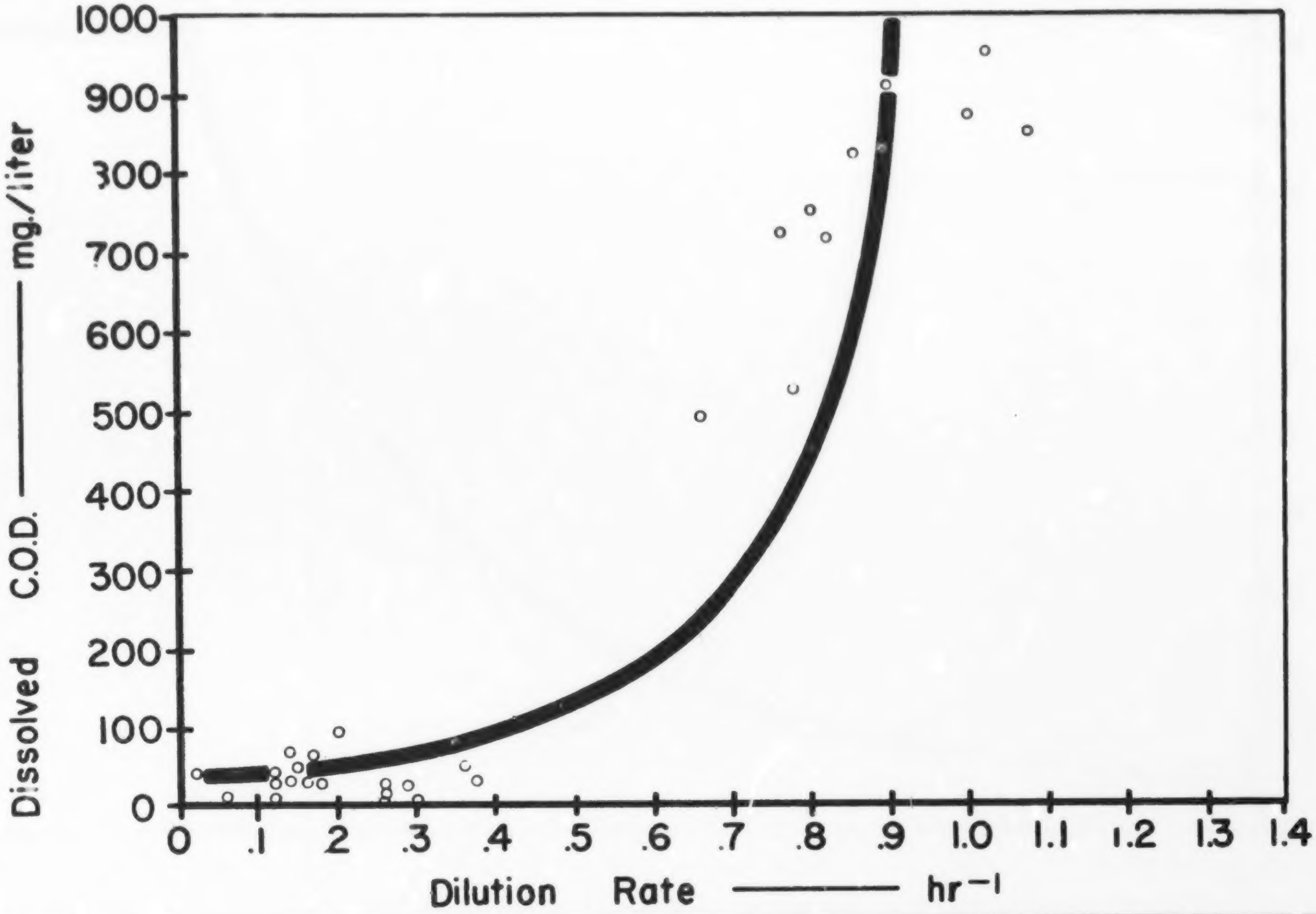


FIGURE 4-3 ——— EFFECT OF DILUTION RATE ON DISSOLVED C.O.D.

CONTINUOUS CULTURE RUN 3

PSEUDOMONAS FLUORESCENS UTILIZING SODIUM ACETATE

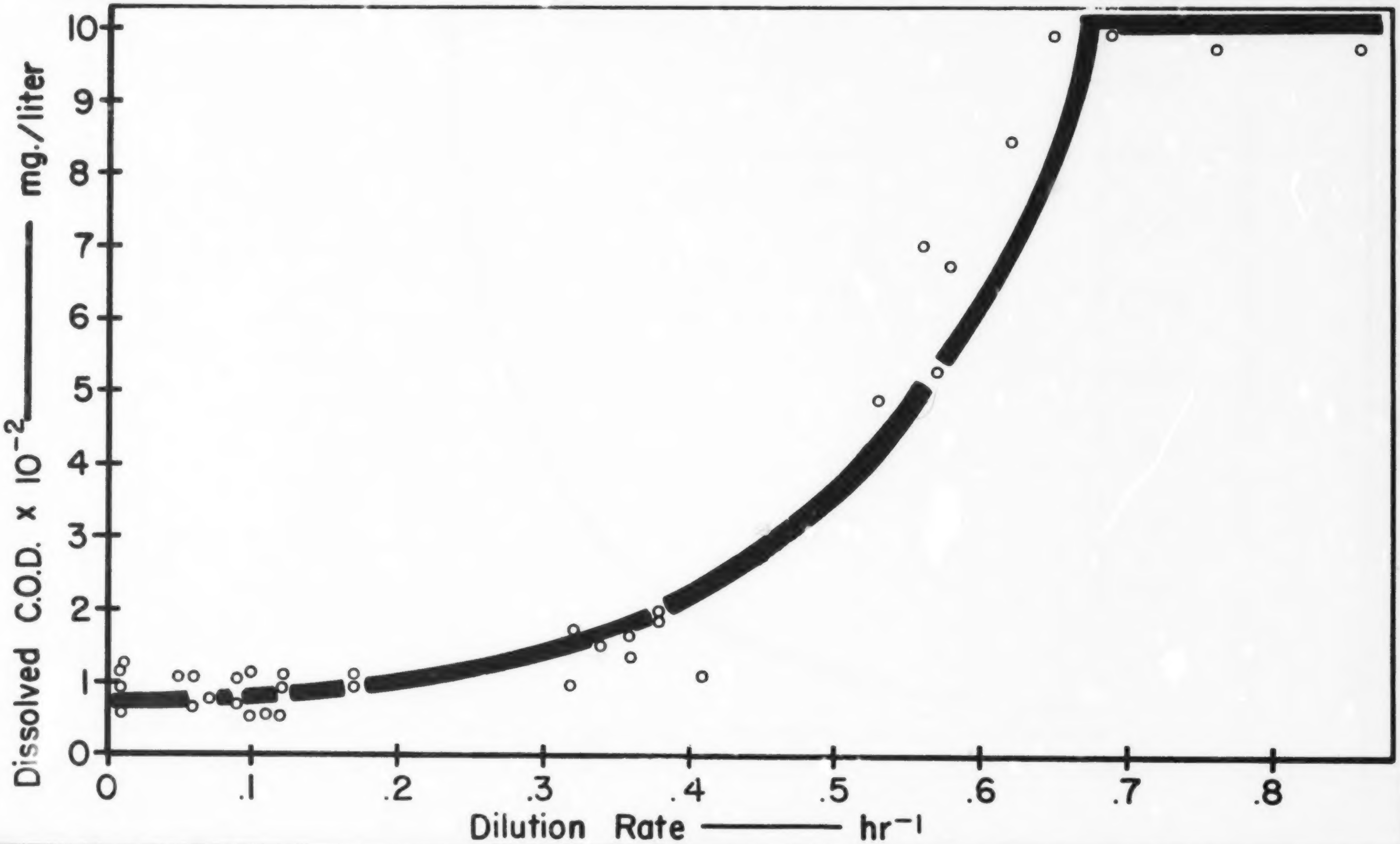


FIGURE 4-4 —— EFFECT OF DILUTION RATE ON DISSOLVED C.O.D.

CONTINUOUS CULTURE RUN 4

ESCHERICHIA COLI B UTILIZING SODIUM ACETATE

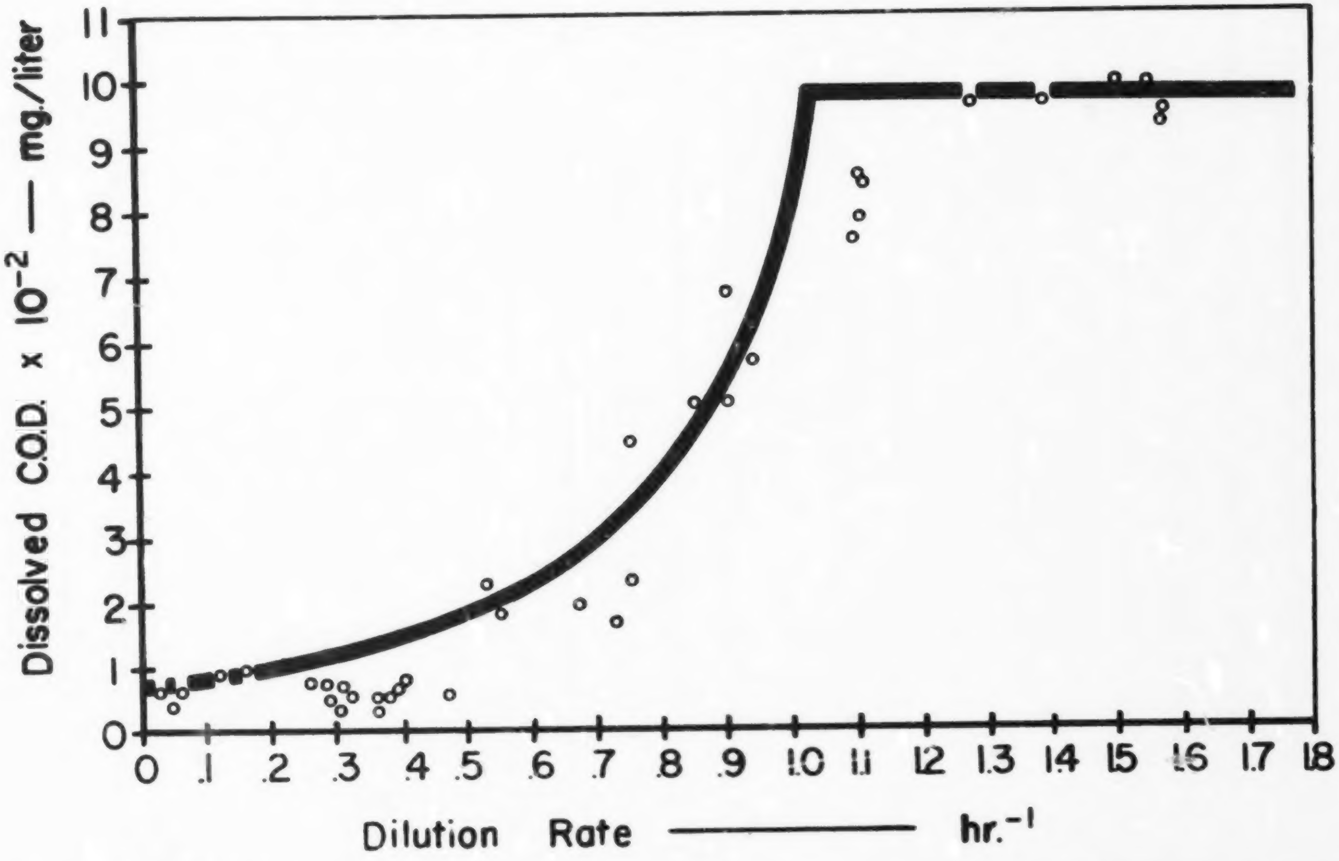


FIGURE 4-5 —— EFFECT OF DILUTION RATE ON DISSOLVED C.O.D.
CONTINUOUS CULTURE RUN 5
ESCHERICHIA COLI B UTILIZING GLUTAMIC ACID

where $y = \frac{S_c' - A}{D}$, $x = S_c'$, $a = \frac{1}{\mu_m}$ and $b = \frac{B}{\mu_m}$. For each run, A was first estimated by extrapolating the lower end of the D vs S_c' curve to the D axis. (It is obvious from Equation (4-9) that $A = S_c'$ when $D = 0$.) With A known, $y = \frac{S_c' - A}{D}$ was calculated. Since $x = S_c'$, it was now possible to calculate the values of a and b by the method of least squares. The values of μ_m and B were then calculated from a and b. The results of these calculations are shown on Table (4-1). For the runs made, A ranged from 33 to 85 mg/l. Even though the reliability of the A values were low because of the means used for their estimation, it is perhaps safe to say that A values do change from one system to another. B ranged from 61 to 89 mg/l which would imply, according to Equation (4-10), that K_s ranged from 100 to 174 mg/l. These values of K_s are somewhat higher than those reported by other researchers using specific substrate concentrations as a measured variable(29)(42)(43)(68). The μ_m, which is a measure of the washout rate of any system, ranged from 0.7 to 1.2 hr.$^{-1}$. This range is typical of organism growing in continuous culture(42)(43)(67).

Finally, correlation coefficients were found for the plot of $\frac{S_c' - A}{D}$ vs S_c'. The correlation coefficients were calculated as 0.94 for runs 2 and 4, 0.96 for runs 1 and 3, and 0.89 for run 5.

DISCUSSION

It has been shown that the effective growth rate is equal to the dilution rate (Equation 4-6). This research has shown that a mathematically definable relationship (Equation 4-7) exists

TABLE (4-1)

Relationships between Dilution Rate and Substrate Concentration

Results of Least Squares Analysis

Run	Organism	Substrate	A*	B*	Max. Growth Rate μm	Correlation Coefficient	Heterogenous Metabolism Rate K_2
1	*P. fluorescens*	Glucose	70	74	0.8	0.96	0.067
2	*P. fluorescens*	Glutamic Acid	33	67	0.7	0.94	0.015
3	*P. fluorescens*	Sodium Acetate	41	61	1.0	0.96	0.031
4	*E. coli B*	Sodium Acetate	85	89	0.8	0.94	0.010
5	*E. coli B*	Glutamic Acid	72	76	1.2	0.89	0.021

$$^{*}D = \mu_m \left(\frac{S_c' - A}{S_c' + B}\right)$$

between D and S_c'. The plotted experimental data was amenable to the use of the equation

$$D = \mu_m \left(\frac{S_c' - A}{S_c' + B}\right) \qquad (4\text{-}7)$$

when S_c' is measured as gross soluble organics as the COD. The equation, which is similar in form to the Michaelis-Menten equation for enzyme reaction rates, has the virtue of simplicity. A better fit or the data might be found by some other function (a parabolic or exponential function might be tried), but the complications in working with a more complex function would limit its usefulness. It follows from Equations (4-6) and (4-7).

$$\mu_e = D = \mu_m \left(\frac{S_c' - A}{S_c' + B}\right) \qquad (4\text{-}12)$$

This equation gives the relationship between the effective growth rate of the bacteria and the dissolved organic concentration in a non-recirculating system at steady state. For the relationship between the true growth rate of the bacteria and the substrate concentration, Equations (4-7) and (4-12) can be combined

$$\mu = K_2 + \mu_m \left(\frac{S_c' - A}{S_c' + B}\right) \qquad (4\text{-}13)$$

The values of K_2 for the systems under study, which were reported previously in Chapter III, have been included in Table (4-1). It will be noted that K_2 ranged from 0.010 to 0.067. Considering the μ_m values for these systems, reference to Equation (4-13) shows that in a steady state system at high process loadings the heterogenous metabolism has only a small effect on the true growth rate, i.e. $\mu = \mu_e$. However, as the process loading decreases, the low substrate concentration reduces growth rate. At such time K_2 may have a large effect on μ_e.

SUMMARY

An equation of the type $\mu_e = \mu_m \left(\frac{S_c' - A}{S_c' + B}\right)$ adequately represents the relationship between the effective growth rate of bacteria and the substrate concentration when the substrate concentration is measured in terms of COD. This function, which is similar to the Michaelis-Menten type, has the virtue of mathematical convenience; it is relatively simple and has the required quantitative properties but it is not in this context the consequence of any definitely formulative hypothesis.

The experimental data indicated that the constants involved in this formulation are unique to each combination of substrate and organism. However, it is felt that the general relationships apply to any predominantly microbacterial process. For an industrial waste treatment process the constants would be different for each waste, and would also be affected by any factor which would change the dominant species of organisms. At present, insufficient data are available to justify the presentation of the range of the coefficients involved.

CHAPTER V - STEADY STATE BACTERIAL CULTURE
CARBON TRANSFER RATES

INTRODUCTION

At this point in the research program, it was evident that the use of pure cultures allowed one to maintain bacteria in a steady state condition. Also, that measure of the gross amount of organic compounds (COD) was adequate to show that the growth parameter, effective yield, does change with process loading. However, the error associated with the COD measurements was sufficiently high to cast some doubt on the validity of the delineation given between yield, synthesis rate, and process loading.

Because of the use of the commonly used field measure of substrate, i.e. COD, it had been necessary to combine all the coefficients in the substrate removed Equation (3-2b) and mass production Equation (3-1a) into overall coefficients. This procedure, although adequate for most engineering purposes, did hamper the scientific advancement of bacterial kinetics. It is only by a delineation of the function of the individual coefficients with a loading parameter that the hypothesized equations for substrate removal and mass production could be validated.

One of the basic difficulties in analyzing the kinetics of bacterial growth in continuous culture, particularly the behavior in the steady state, is that the parameter normally monitored to observe the functioning of the biological system remaining constant with time. These environmental circumstances indicate the possibility of introducing a supply of radioactive substrate during periods of steady state operation. This procedure, which was followed in this Chapter, allowed the measurement, under steady state conditions, not only the time variation of the normally external parameters but also the internal transfer rates, i.e. the transfer of carbon into and out of the cells. Carbon measured by the Van Slyke apparatus was chosen as a more accurate measurement of the gross substrate, mass of cells, etc. The balance on the systems were then measured as equivalent carbon rather than oxygen.

THEORETICAL CONSIDERATIONS

If E_i is designated as the amount of carbon in any compartment (i), at any time (t), for each compartment a balance of transfer rates in and out of the compartment may be written

$$\frac{dE_i}{dt} = \sum_j \rho_{ji} - \sum_j \rho_{ij} \qquad (5\text{-}1)$$

where j is the number of compartments interacting with compartments i.

Similarly, if at some time a quantity of radioactive tracer material is placed within the system, it will be distributed among the various interacting compartments within the system and the rate of charge of radioactivity in a compartment (i) is given by

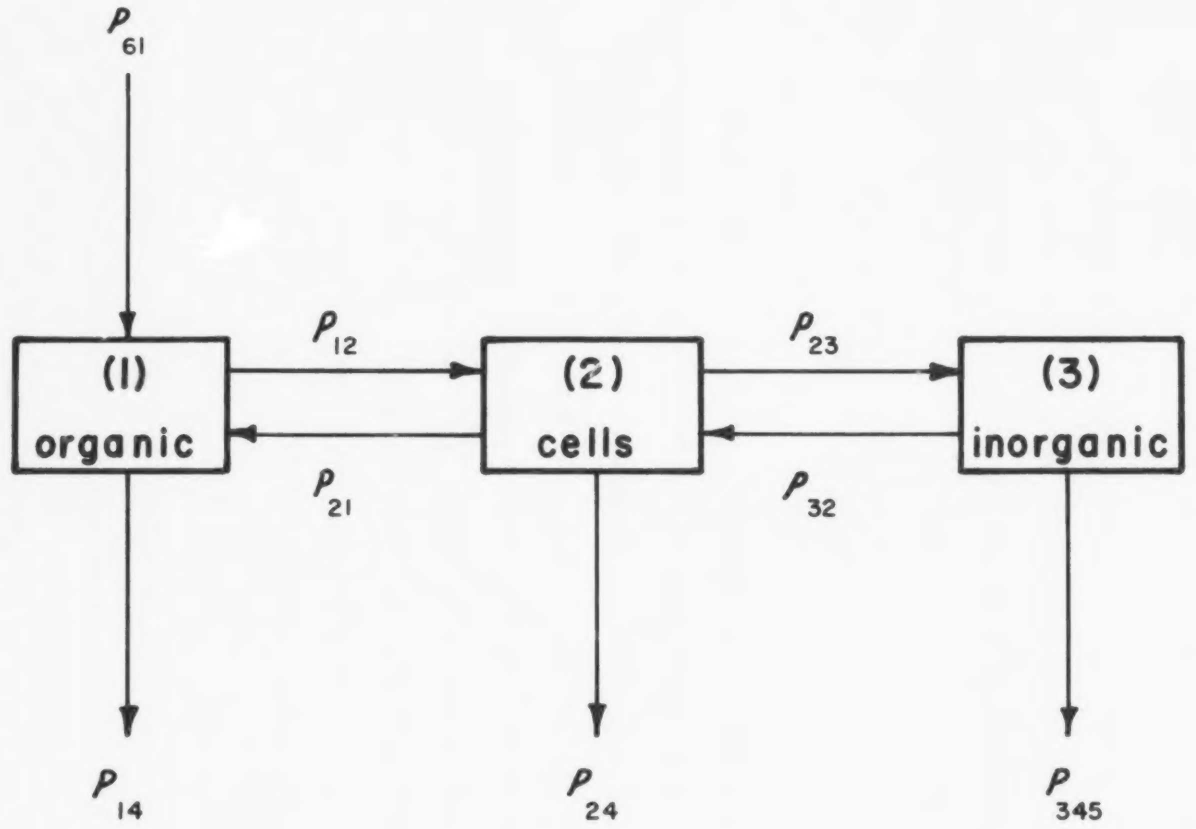
$$\frac{dR_i}{dt} = \sum_j \rho_{ji}\, a_j - a_i \sum_j \rho_{ij} \qquad (5\text{-}2)$$

where R_i is the quantity of radioactive tracer in convenient units (e.g., microcuries of nanocuries), and $a_{i,j}$ is the specific activity or radioactivity per unit weight of carbon. It can be shown that the total amount of activity in any compartment is expressed as

$$R_i = E_i\ a_i \qquad (5\text{-}3)$$

In the system under study, the inorganic, soluble organic and suspended carbon were measured. With these measurements the continuous culture can be thought of as a six compartment closed system. This would allow the writing of two sets of six simultaneous equations(31). A simpler alternative suggests itself since, due to the layout of the system, three of the compartments are not conservative during steady state. This leaves a three compartment system as schematically shown in Figure 5-1. It can be seen that the feed compartment 6 continually decreases. The liquid compartment 4 is separated from the aeration unit such that the material once passed into the effluent cannot return. Also, due to the physical arrangement, the gas effluent was passed through sodium hydroxide to retain the carbon dioxide in compartment 5. The The rate of overall loss of carbon dioxide $\rho\ 345$, which is the sum of $\rho\ 34$ and $\rho\ 35$, was measured by a time analysis of compartment 5. Thus, an open three compartment system as shown in Figure (5-1) was used.

For the three steady state ($dE_i/dt = 0$) compartments chosen to represent the carbon kinetics in the chemostat unit, Equation (5-1) may be written as follows:



P_{61}
(1) organic
(2) cells
(3) inorganic
P_{12}
P_{21}
P_{23}
P_{32}
P_{14}
P_{24}
P_{345}

FIGURE 5-1

THE OPEN 3 COMPARTMENT SYSTEM

$$\rho_{61} + \rho_{21} = \rho_{12} + \rho_{14} \tag{5-4a}$$

$$\rho_{12} + \rho_{32} = \rho_{21} + \rho_{23} + \rho_{24} \tag{5-4b}$$

$$\rho_{23} = \rho_{32} + \rho_{345} \tag{5-4c}$$

As a result of the fact that the system is in a steady state, there is another relationship which can be written

$$\rho_{61} = \rho_{14} + \rho_{24} + \rho_{345} \tag{5-5}$$

However, Equation (5-5) is not independent but rather the result of the addition of the Equations (5-4) together.

Since specific activity (2) rather than amount of radioactivity (R) is usually measured experimentally, it is advantageous to have the Equation (5-2) expressed in terms of rate of change of specific activity with time rather than amount of activity with time. If the integrated form of the specific activity rate equations can be obtained, they will describe completely the variations in the system with time. Differentiating Equation (5-3) with respect to time gives

$$\frac{dR_1}{dt} = a_1 \frac{dE_1}{dt} + E_1 \frac{da_1}{dt} \tag{5-6}$$

Substituting for dR_1/dt into the left hand sides of Equation (5-2) individually and subtracting the result from a_1 multiplied times Equations (5-4) for each compartment yields (for radioactivity instantaneously injected into compartment 1, i.e., $a_6 = 0$),

$$\frac{dR_1}{dt} = \frac{E_1 da_1}{dt} = (a_2 - a_1)\,\rho_{21} - a_1\,\rho_{61} \tag{5-7a}$$

$$\frac{dR_2}{dt} = \frac{E_2 da_2}{dt} = (a_1 - a_2)\,\rho_{12} + (a_3 - a_2)\,\rho_{32} \tag{5-7b}$$

$$\frac{dR_3}{dt} = \frac{E_3 da_3}{dt} = (a_2 - a_3) \quad \rho_{23} \qquad (5\text{-}7c)$$

There are, in the case of this three compartment system, six independent equations. There are eight ρ values, four of which are directly measurable and readily known. Choosing two of the known ρ values and calling them unknowns enables the solution of resulting six unknown ρ by a matrix.

The tangent to the specific activity vs. time curve must be obtained for the graphical determination of da_1/dt. This procedure has inherent error. Note that for a complete solution for the set of ρ values, it is necessary to have only one set of experimental data at some time, t. To determine the da_1/dt graphically with any degree of accuracy, however, requires a reasonably complete time curve of specific activity.

Using this procedure and some preliminary data, the system of simultaneous equations (5-4) and (5-7) have been solved by matrix inversion on the IBM 1410 digital computer and it was shown that a small error in da_1/dt causes a large error in the solution of the ρ values. Some of the error in the ρ values was also due to the fact that the data used rendered the system of equations somewhat ill-conditioned. The experimental a_1 values were too close together since they were obtained late in the process with time. Useful data can only be collected when the continuous culture is in radioactive non-equilibrium, i.e. when the specific activity in all compartments is changing.* When all

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*The system used to collect preliminary data was fed radioactivity continuously, in which case all da_1/dt begin to approach zero after some time has elapsed. All a_1 approach the specific activity of the feed.

da_i/dt are equal to zero (radioactive equilibrium), solution for the transfer rates is not possible (except that all $\rho_{ji} = 0$).

EXPERIMENTAL PROCEDURES

Materials and Apparatus

The description of the continuous culture apparatus which was used, including the reaction vessel, feeding system, and air supply, has been discussed in Chapter III. Modifications to this apparatus were made to include an influent sodium hydroxide trap to remove carbon dioxide from the incoming air, and a modified effluent collection system. The modified effluent collection system incorporated a closed 2,000 ml. capacity separatory funnel, effluent sodium hydroxide trap and barium chloride trap in series. Also, a sampling tube was added, preceded and followed by three-way stopcocks to permit collection of samples of liquid effluent at various times during a run.

The separatory funnel was fitted with ground glass at the top permitting the entrance of the composite gas-liquid effluent from the reactor and exit of gas effluent to the hydroxide trap. A stopcock at the bottom of the funnel permitted the removal of the liquid effluent at the end of each time period to form the composite effluent (compartment 4) without disturbing the effluent gas flow.

Following the separatory funnel was a liquid trap to prevent the flow of liquid effluent from the separatory funnel to the hydroxide trap or vice versa. Following the liquid trap was a 1,000 ml. aspirator bottle (normally containing 500 ml. of 2 normal sodium hydroxide) permitting sampling of the trap contents (compartment 5) at various intervals without disturbing the gas flow. The hydroxide trap was followed by a barium chloride trap of 200 ml. capacity. Gas effluent from the final barium chloride trap was permitted to escape to the constant temperature room atmosphere. The trap system was found to be 93 - 98% efficient in the collection of effluent carbon dioxide from the apparatus. It was decided on this basis that the incurring of additional head loss and time consumed in measuring two samples at each time period, outweighed the advantages of a second hydroxide trap in series with the first.

The head loss through the modified effluent system was measured 9 to 9.5 inches of water. It was found that the greater the head loss through the effluent system, the greater are the variations in feed and effluent flow rates. Operation of the apparatus at low feed rates was also more unstable. As can be seen from the data, steady state (no change in carbon concentration) can readily be maintained within the continuous culture apparatus.

The media compositions and the history of *Pseudomonas fluorescens* and characteristics of the strain used are the same as those given in Chapter III.

Analytical Procedures

The VS wet combustion technique was used for the determination of organic carbon. The procedure followed was that of Van Slyke and Folch(72) with only a few minor alterations. The determinations of inorganic carbon was done by a modification of the VS technique.

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It was necessary to determine inorganic carbon (CO_3, HCO_3 and CO_2) in the liquid portion of the culture (compartment 3) and in the sodium hydroxide effluent gas trap contents (compartment 5). Samples were collected from the centrifuged culture supernatant, and from the sodium hydroxide trap, placed in VS reaction tubes, tightly stoppered, and stored in the liquid state in the refrigerator until analysis. Storage at the decreased temperature favored uptake of atmospheric CO_2 rather than loss from the sample. For analysis alundum crystals were placed in the sample tube, the top of the tube was ringed with phosphoric acid and the tube placed on the VS unit. Lactic acid (2N) was added to the tube after evacuation. The mixture was then heated to release the CO_2 from the sample and the gas collected as in the combustion procedure.

Carbon analyses were performed to determine the standard curve for combustions. These values include the range of actual values normally observed during the runs. The results listed in Appendix Table (C-3) would seem to indicate that the absolute value of the deviations are independent of the quantity of carbon in the sample analyzed. This observation was similarly made by Van Slyke, Page, and Kirk(71) and Van Slyke and Folch(72). The percent deviation will vary depending on the quantity of carbon in the sample.

There is insufficient data to draw a conclusion regarding the frequency distribution of the carbon measurements. It was assumed that the distribution is normal. The same assumption applies to other random variables discussed in the study (for example, specific activity, rate of change of radioactivity within a compartment, etc.). It is further assumed that the final result, namely carbon transfer rate, is a random variable and, thus, normally distributed. When additional data become available using this technique, these assumptions may be revised.

The preparation of barium carbonate cakes for counting and the radioactivity measurement procedure are discussed by Washington(73).

OPERATIONAL PROCEDURES

Procedure for Radioactive Glutamic Acid UL-Carbon-14 Study

Summary - An active culture of *Pseudomonas fluorescens* metabolizing at the desired flow rate was maintained within the continuous culture apparatus. Capital letters throughout this section will refer to parts of the apparatus as illustrated in Figure 3-3.

A quantity of radioactive uniformly labeled-glutamic acid-carbon-14 was injected through the rubber hose into the reactor at D. This was time "zero" of a given run. After injection, samples were taken for carbon analyses and radioactivity counting of compartment Numbers 1, 2, 4 and 5 at elapsed times of 5, 30, 60, 90, 120, and 150 minutes. Thus, changes in size of compartment (quantity of carbon present) with dilution rate and changes in specific activity could be monitored for two and one-half hours after introduction of tracer. With these data it was possible to determine the carbon transfer rates between compartments at different dilution rates.

The Stock Culture - A stock culture of *Pseudomonas fluorescens* utilizing glutamic acid was maintained at a low dilution rate in a special chemostat unit from May to October 1964. The original inoculum was obtained from Dr. H. Ehrlich of the Biology Department at Rensselaer Polytechnic Institute. This culture was checked frequently by visual observation and gram stain.

A separate culture vessel was inoculated from this stock culture for each run.

Inoculation of New Culture - A portion of 100 ml. of inoculum taken from the stock culture (section 1b) was collected in sterilized test tubes and transferred to the experimental reactor vessel. This was performed when the new reactor was engaged with the influent air line and it contained approximately 300 ml. of feed solution. Any leaks were, therefore, positive outward. The reactor containing the inoculum was disengaged from the feed bottle and the culture was permitted to grow for 10 - 15 hours without feeding (batch). The culture was observed visually under the microscope before and after inoculation.

At the end of this growing period, continuous feeding was begun and the flow adjusted to the desired rate. The culture was allowed to proceed at the given rate for 20 - 24 hours before injection of radioactivity, to ensure a steady state condition within the reactor.

Measurement of Compartment 3 - In an initial run, it was discovered that meaningful results could not be obtained for specific activity measurements within compartment 3, due to the very small amount of carbonate, bicarbonate and carbon dioxide present in the culture vessel at any instant during the run. Van Slyke blank determinations, in most cases, exceeded 50% of the total value observed for a sample. Larger sample sizes were limited by the capacity of the Van Slyke (VS) unit and the limitation imposed by the possibility of upsetting the steady state. Fifteen milliliters of sample were taken at each time interval (1.5% of the total volume). A maximum of 4 milliliters could be analyzed in the VS unit coincident with the requirement of acid addition to release the inorganic carbon.

Storing samples in the refrigerator until the following day further aggrevated the problem.

An analysis of the amount of carbon in compartment 3 was performed, therefore, before injection of the tracer, but after steady state had been achieved. Usually this sampling occurred one or two hours before beginning the run. The sample was collected and analysis immediately performed on an aliquot of whole effluent (containing cells). This observation was used as the size of compartment 3. The specific activity of compartment 3 was calculated from compartment 5 data, as explained below.

Injection of Tracer - When it was determined that a steady state existed and preparation for initiation of the run were completed, the tracer was injected. One milliliter of:

New England Nuclear Corp; NEC-290
L-Glutamic Acid-C-14 (uniformly labeled)
Specific activity 1.393 mc per mg,
Lot. No. 120-200-96, in 0.01 N HCl

containing about 0.1 millicuries was injected into the reactor vessel at D. The injection was accomplished using a B-D Yale-C-5096 hypodermic syringe with a 2 milliliter capacity. This was time zero for the run.

Instantly all of the activity was present in the soluble-substrate compartment 1.

Collection of Samples - Samples were taken after elapsed times indicated above by engaging the two three-way stopcocks at E and H and permitting the effluent to bypass collection vessel C into the VS reaction tube mounted at G. While the VS tube was filling, a sample was taken from the effluent NaOH trap (compartment 5). The liquid effluent was drained to a collection vessel (final effluent) and samples were taken of the resulting composite at each time interval (compartment 4).

When the VS tube was filled, the bypass was disengaged so that the combined liquid-gas effluent could again follow the normal route.

Collection of all samples required about three minutes.

Fifteen ml. of total sample were collected at each interval in the VS tube. Five ml. of this liquid (containing compartments 1, 2 and 3) were immediately placed in 0.5 ml. of $HgCl_2$ to make approximately 0.001 M mercuric ion to inhibit further metabolism by the organisms. The remainder was discarded.

After each sample collection (C was empty), $HgCl_2$ solution was injected into C at J using a B-D Yale hypodermic syringe with a 10 ml. capacity. This inhibited metabolism from continuing within vessel C and consequently prevented the resulting CO_2 from being collected in the final NaOH trap. Thus, carbon dioxide collected in the trap was primarily that which resulted from metabolic activity within the culture vessel itself.

Disposition of Samples - After collection, the sample from the sodium hydroxide trap was immediately pipetted into a VS tube, tightly stoppered, and placed in the refrigerator to prevent loss of CO_2 from the sample.

The liquid sample (compartments 1, 2, and 3) was placed in a centrifuge tube containing mercuric chloride and centrifuged.

Centrifuge: International Model HT
Head No. 856; Max. rpm 17,000
Cellulose-nitrate tubes - 15 ml. capacity

All samples were centrifuged at approximately 12,000 rpm or about 17,000 x G for five minutes. It was necessary to complete handling of the sample to the washing-of-cells stage within the 30 minute period between samplings. This included the time required for the centrifuge to reach 12,000 rpm and slow to a stop.

After centrifugation of the sample, compartment 1 was carefully pipetted from the supernatant in the centrifuge tube, placed in a VS reaction tube, and into the oven to dry (approx. 90°C). The remainder of the supernatant was discarded and the sediment was suspended in distilled water to wash the cells. This mixture was centrifuged at the same time as the sample from the succeeding time period. After washing, the supernatant was discarded, the cells resuspended, carefully washing down the sides of the tube and the whole mixture was transferred to a VS tube and then dried in the oven. This sample was compartment 2, the cells.

After the sample from the trap (compartment 5) and the liquid effluent sample (compartments 1, 2, and 3) were disposed of, a sample from the final effluent (compartment 4) was taken from the composite of all previous time periods, placed in a VS tube and dried in the oven.

This concluded the sample taking for a given time period.

Procedure for Radioactive Sodium Bicarbonate-C-14 Study

This procedure is substantially the same as that using glutamic acid as a tracer except as discussed below.

The use of tagged sodium bicarbonate results in all of the radioactivity being present instantaneously in compartment 3 rather than compartment 1 at time zero. It was believed desirable to check the applicability of the mathematical model to more than one tracer system. The use of sodium bicarbonate C-14 afforded this opportunity and at the same time permitted a comparison of the numerical values of carbon transfer rates obtained by two techniques.

The inoculation of a new culture proceeded in the same fashion as described previously. Measurements for compartment 3 were taken during the run.

Injection of Tracer - When steady state had been achieved, the injection of two milliliters of

New England Nuclear Corp., NEC-86H,
Sodium bicarbonate C-14,
Specific activity 0.238 mc per mg,
in 5 ml. of distilled water plus NaOH
(pH = 8.0)

containing about 0.2 mc, was performed with the 2 ml. capacity syringe, calibrated in 0.1 ml.

Collection of Samples from the Apparatus - Collection of samples from the reaction vessel and from the hydroxide trap proceeded as in the other runs.

Disposition of Samples - Disposition of samples during this run was the same except that compartment 3 was monitored along with the others. Two 5 ml. samples of reactor effluent were centrifuged and compartment 1 sample was taken from one and compartment 3 from the other. Each was 3 ml. in size.

The compartment 3 sample was kept tightly stoppered in the refrigerator similar to the compartment 5 sample, until analysis. The VS procedure for inorganic carbon was subsequently carried out on each sample.

RESULTS

For clarity, the experimental results are presented in three sections: (A) Peripheral transfer rates, which can be calculated independently of the radioactivity data, and (B) compartmental specific activity variations which are used to calculate (C) internal transfer rates. The estimation of error in each case is presented within the section.

The runs were performed at the following dilution rates (D):

Run Number	D
11	0.42
12	0.50
15	0.51
13	0.54
10	0.59

Peripheral Transfer Rates

The peripheral carbon transfer rates (see Figure (5-1)), namely the feed rate (ρ_{61}), the carbon loss to the effluent in liquid state (ρ_{14}, ρ_{24}, ρ_{34}) and carbon dioxide (ρ_{35}) can be calculated from the carbon analyses data obtained during a run. The dilution factor (D) is known for each run since the flow is set. Also, E_6, E_1, E_2 and E_3 were measured carbon values. The transfer rate ρ_{35} is equal to the rate of accumulation of carbon in the effluent sodium hydroxide trap. Carbon measurements were made of the trap contents at intervals during the run. The measured carbon values for compartments 1 and 2 are presented in Appendix Tables (C-1) and (C-2), respectively. The standard deviation and the probable error estimates are presented with these data.

Knowing the dilution rate value for a given run and the measured carbon values, the peripheral transfer rates can be calculated from the following expressions:

$$\rho_{61} = DE_6 \tag{5-8a}$$

$$\rho_{14} = DE_1 \tag{5-8b}$$

$$\rho_{24} = DE_2 \tag{5-8c}$$

$$\rho_{34} = DE_3 \tag{5-8d}$$

An exact carbon balance was obtained by Equation (5-5)

$$\rho_{61} = \rho_{14} + \rho_{24} + \rho_{345} \qquad (5\text{-}5)$$

the difference between influent carbon (ρ_{61}) and the sum of effluent carbon in the liquid state (ρ_{14}, ρ_{24}, ρ_{34}) and effluent carbon in the gaseous state (ρ_{35}) was distributed to all the observed values by weighting according to the absolute values of the ρ's. These prorated transfer rates then formed a balanced system with regard to inflow and outflow of carbon. These values were later used with the weighted means of the internal transfer rates obtained from radioactivity analyses to obtain a balance internally.

The peripheral transfer rate values calculated as discussed above and their associated standard deviation are presented in Appendix Table (C-6). The interrelationship of their variations is more apparent in Figure (5-2). It is seen in Figure (5-2) that, although some of the standard deviations are large, the data appear to be quite good for a biological system. The cells to effluent transfer rate (ρ_{24}) seem to reach a maximum which corresponds to the maximum effective yield coefficient observed. This latter will be discussed below. This maximum ρ_{24} is not necessarily associated directly with the maximum yield.

Variations of Specific Activity with Time

The specific activity of a compartment i, at any given time is given by $a_i = R_i/E_i$. The count rate was converted to R_i by the use of the observed standard curve values, divided by the amount of carbon in the given sample. The above relationship was used to

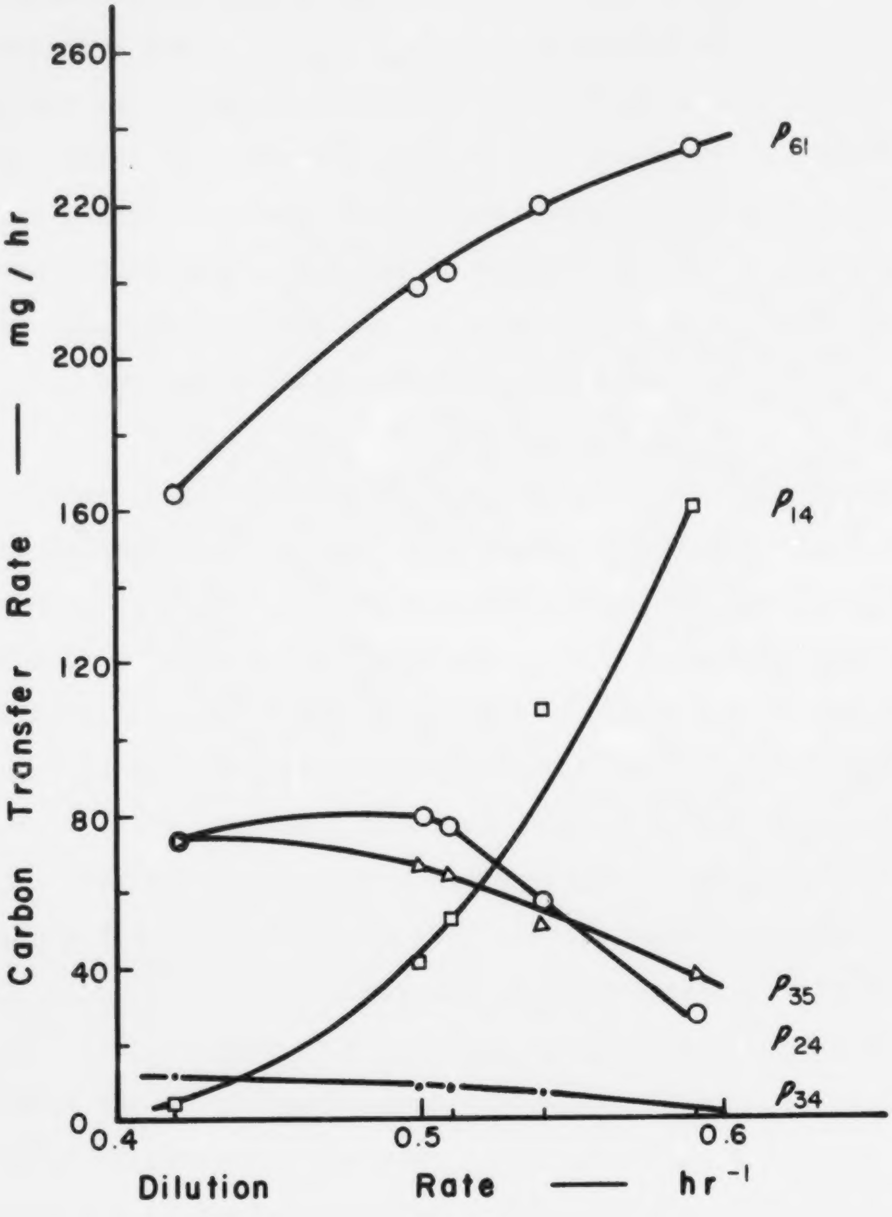

FIGURE 5–2

PERIPHERAL CARBON TRANSFER RATES

calculate the specific activity value for a sample at a given time during a run using the particular sample. To plot curves of R_1 versus time from which dR_1/dt values were obtained, the average value of E_1 was used to calculate back.

All values of specific activity are expressed in nanocuries per milligram of carbon (nc/mg) and radioactivity in nanocuries (nc). Appendix Tables (C-3) and (C-4) contain the specific activity values and the associated probable error for compartments 1 and 2, respectively.

The specific activity values for compartment 3 were obtained from data of compartment 5, except for run no. 15 in which the activity was believed high enough to be measured with some reliability. The rate of change of activity in compartment 5 is given by

$$\frac{dR_5}{dt} = \rho_{35}\, a_3 \qquad (5\text{-}2a)$$

The values of carbon-12 and carbon-14 were measured experimentally, thus permitting the calculation of a_3 at various times throughout the runs. Appendix Table (C-5) gives the specific activity values for compartment 3 with associated probable error. It should be noted that the values given for ρ_{35} are measured values and not the final adjusted values as discussed previously. This was done because activity measurements of the contents of the barium chloride trap were not made.

All values obtained experimentally for specific activity within compartment 2 were adjusted to take into account the viable portion of the cells of compartment 2. The percent viability for

Pseudomonas fluorescens growing on glutamic acid in the same continuous culture apparatus had been determined previously and reported in Chapter III of this study. The percent viability was found to be constant at a value of 56 percent. It should be pointed out that both the slide culture technique and spot plate techniques which were used to determine percent viability are based on organism reproduction. These do not take into account the cells which, for one reason or another, have lost the ability to reproduce, but which remain biochemically active.

The fact that a non-viable portion exists within compartment 2 ostensibly introduces an inhomogeneity in this compartment. Figure (5-3) schematically illustrates a possible interpretation of what occurs. If it is assumed that only viable cells can move carbon in and out of the compartment as illustrated, then the non-viable portion could be ignored as merely a non-reactive mass present in the system. This is true with regard to the overall metabolic activity of the systems as it is in a steady state. The movement of radioactivity, however, is not at steady state; if it were, no useful data could be collected. Early in any given run, the specific activity of the viable portion is very low; and, therefore, the amount of radioactivity moving across the boundary into the non-viable portion is small. Later, as the specific activity increases, the quantity of radioactivity moving across the boundary increases. Even though cells are always being carried out in the effluent, this phenomenon has the overall effect of increasing the quantity of radioactivity retained in the non-viable

Diagram Showing Movement of Radioactivity to and Within Compartment 2 (Cells)

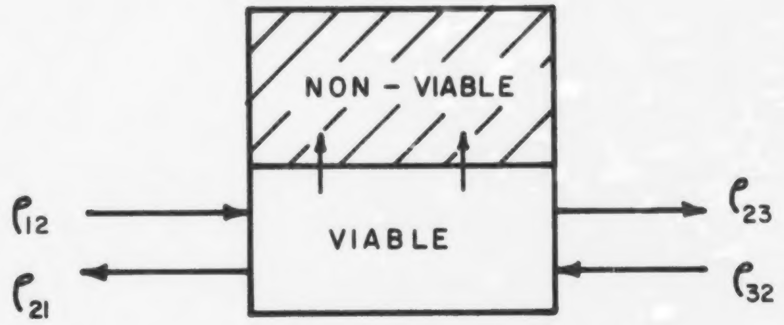
NON - VIABLE
VIABLE
ρ_{12}
ρ_{21}
ρ_{23}
ρ_{32}

FIGURE 5-3 - COMPARTMENT 2 INSOLUBLE CARBON INHOMOGENEITY

portion. Since the total amount of carbon remains constant, this would be observed as a slightly higher value of specific activity in compartment 2.

Run No. 15 differs from the others in that radioactivity in the form of sodium bicarbonate C-14 was injected into compartment 3 instead of compartment 1.

The specific activity variations of compartment 1, the initially labeled compartment in runs Nos. 11 through 14, are summarized in Figure (5-4) which is a semilog plot of specific activity versus time. It is seen that as dilution rate increases, the curves of a_1 approach a single exponential expression, or in other words, approach a straight line. The curve for Run 11, corresponding to the lowest feed rate, is a multi-exponential curve.

Internal Transfer Rates

The internal transfer rates are ρ_{12}, ρ_{23}, ρ_{21}, and ρ_{32}. In general, four of the available equations have been used to calculate the unknowns and the other two checked by substitution of the resulting transfer rate values. Specifically, Equation (5-7a) has been used to determine ρ_{21}, and Equation (5-7c) has been used to calculate ρ_{23}. After determining ρ_{21} and ρ_{23}, ρ_{12} and ρ_{32} were calculated using Equations (5-4). These values and their associated standard deviations are presented in Appendix Tables (C-7) and (C-8). A weighted mean was then computed by minimizing the variance and a standard deviation of the weighted mean was calculated. These statistics are included with the final reported values of internal transfer rates.

Values of transfer rate calculated during a given run are not equally good. This is due to the variation in error in

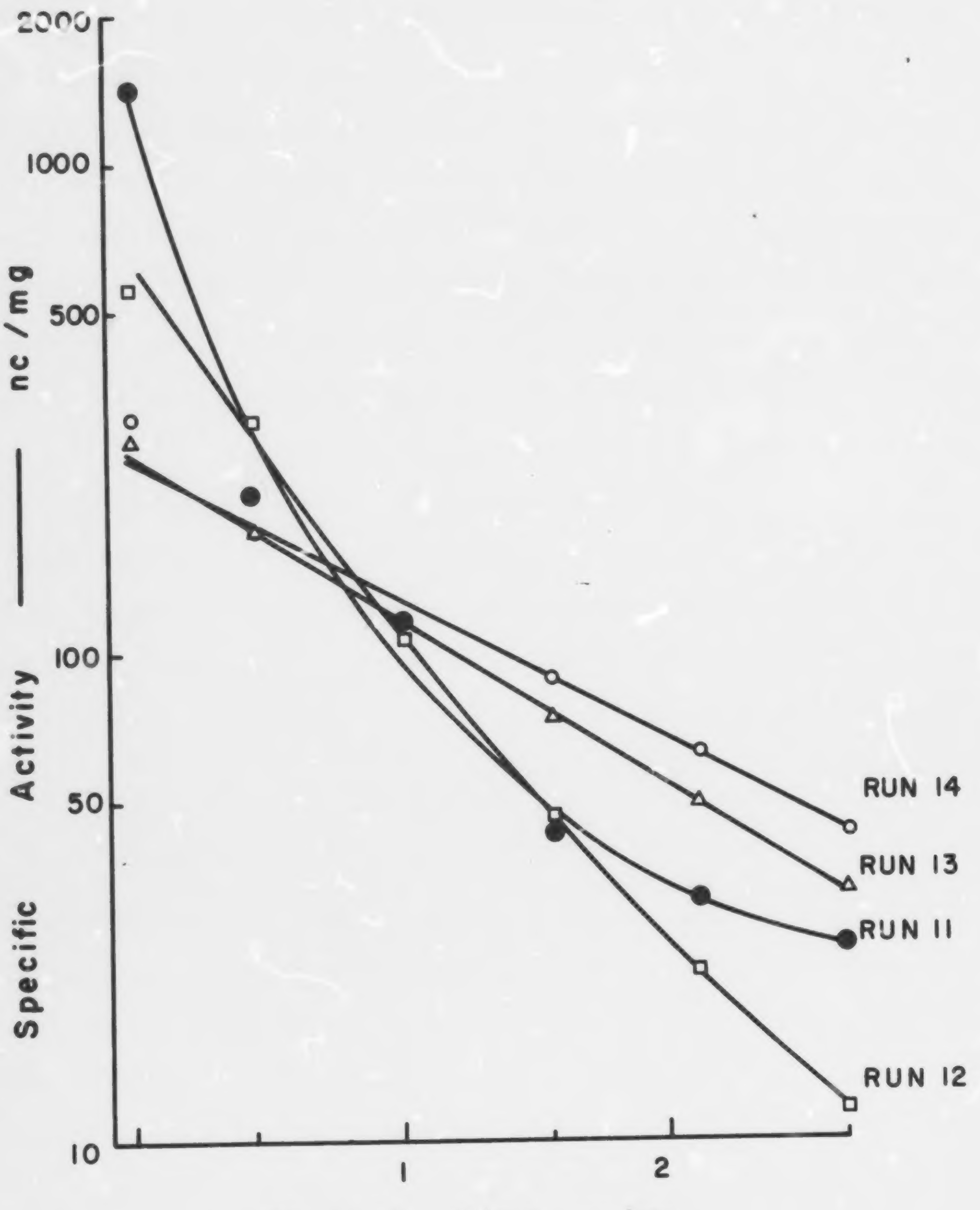

FIGURE 5-4 -COMPARTMENT 1-SOLUBLE ORGANIC CARBON - SPECIFIC ACTIVITY

determination of slopes and variations in absolute values of differences in specific activity used in calculating the transfer rates. A weighted mean of the calculated values of ρ_{21} and ρ_{23} was determined, therefore, using the values in Appendix Tables (C-7) and (C-8) and the calculated standard deviations. These values are presented in Appendix Tables (C-9) and (C-10) along with the raw mean for comparison of consistency. The carbon transfer rates for ρ_{12} and ρ_{32} together with their error are given in Appendix Table (C-11). The resulting internal transfer rate values are plotted on Figures (5-5) through (5-8).

As mentioned previously, it was necessary as a final step to substitute the calculated transfer rate values back into the equations of compartment 2 to check the mathematical model hypothesis. As a final checking step, it was decided to use Equation (5-9) which includes external transfer rates as calculated independently of the radioactivity data.

$$\frac{dR_1}{dt} + \frac{dR_2}{dt} + \frac{dR_3}{dt} = -\rho_{14}a_1 \; -\rho_{24}a_2 \; -\rho_{345}a_3 \qquad (5\text{-}9)$$

This equation also includes all three measured values of specific activity and slope of radioactivity. Appendix Tables (C-12) through (C-16) show the observed balance of Equation (5-9) -- left hand side (LHS) versus right hand side (RHS). In words, Equation (5-9) states that the rate of change of radioactivity internally equals the rate of change of radioactivity externally.

DISCUSSION

Mixing

It is apparent that significant short circuiting of the carbon-14 tracer directly into the effluent or incomplete mixing

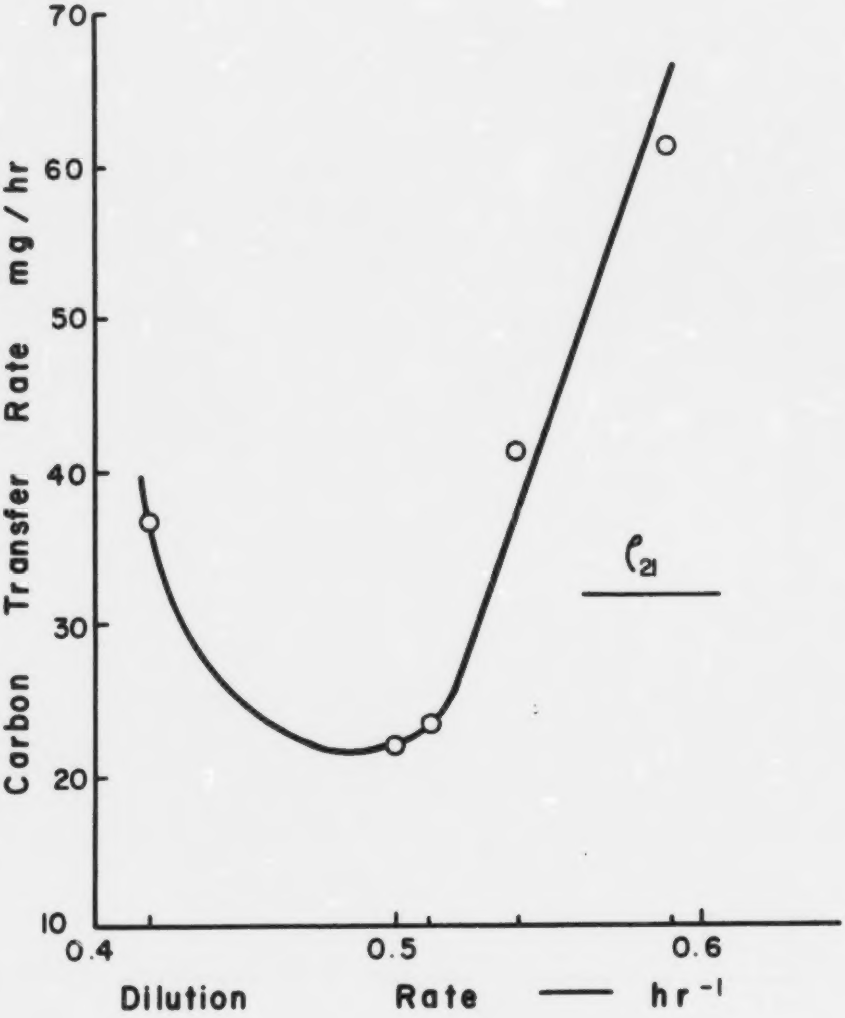

FIGURE 5-5 - CELLS TO SUBSTRATE CARBON TRANSFER RATES

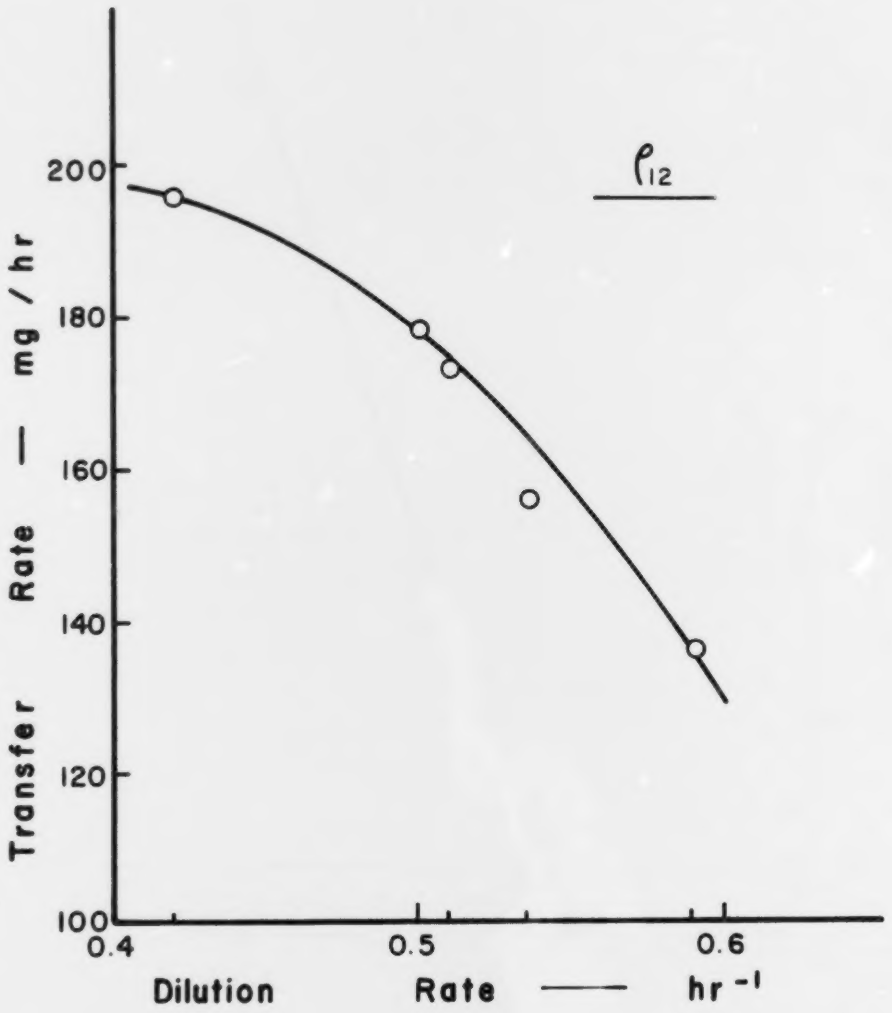

FIGURE 5-6 SUBSTRATE TO CELLS CARBON TRANSFER RATE

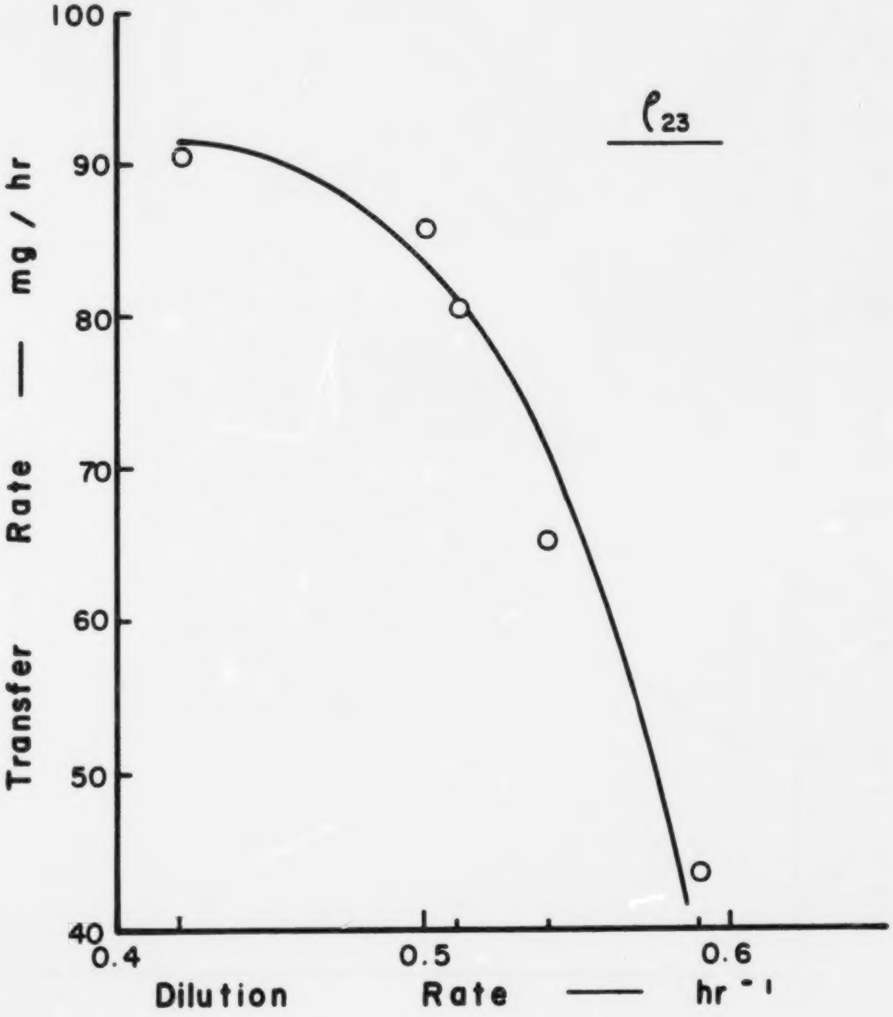

FIGURE 5-7 CELLS TO INORGANIC CARBON TRANSFER RATE

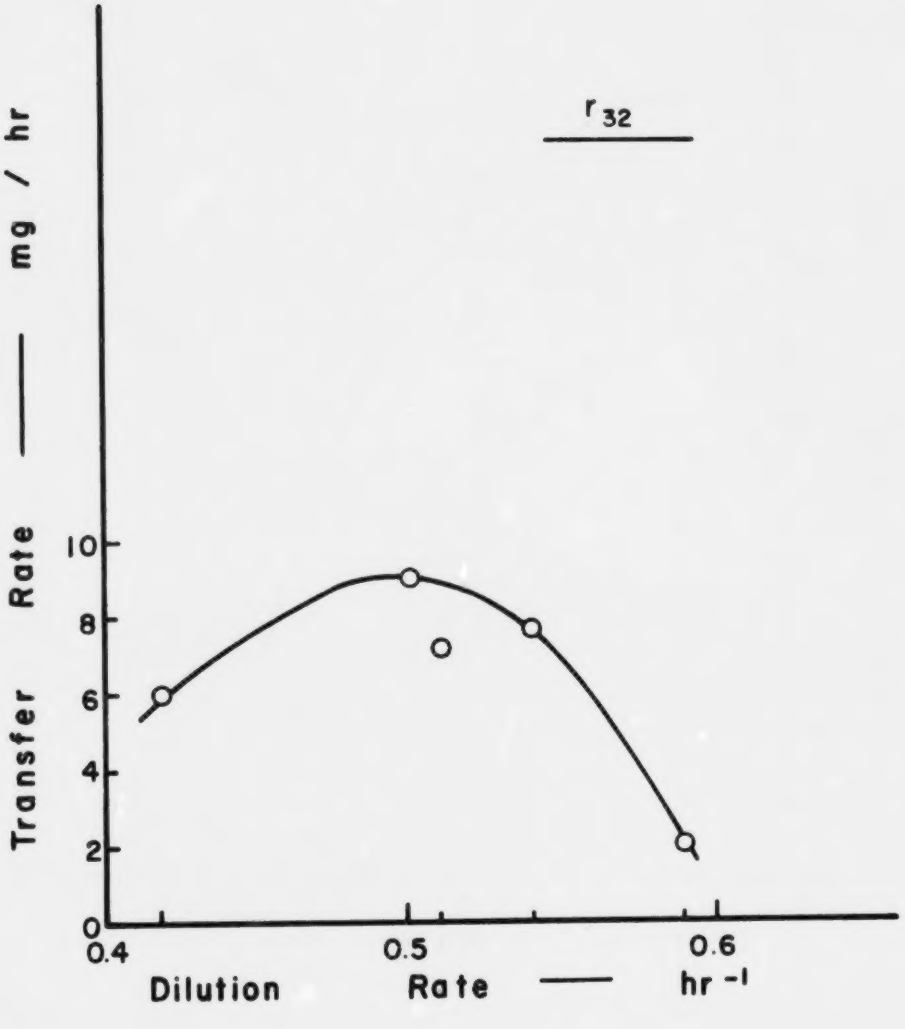

FIGURE 5-8 INORGANIC TO CELLS CARBON TRANSFER RATE

will affect the results of the analyses. The technique, as described, assumes instantaneous mixing of the contents of the reactor. Dyes were used in the experimental arrangement shown in Figure (3-3) and no observable time lapse occurred between injection of the dye and complete coloring of the solution. No stagnant zones were observed.

Pipes and Grieves, et al.(17)(18)(49()50), have done a great deal of work on the effect of stagnant zones and incomplete mixing in aeration vessels. They used inert tracers such as NaCl and measured chlorides at various points in the reaction vessels. They have presented data which permit a gross estimate of the magnitude of the effects of these parameters on this system. Calculations based on Pipes' analysis showed that the stagnant zone of the apparatus used in this study would be essentially zero and that short circuiting would be a maximum of 6 percent of the total flow. If the flow rate contributes significantly to the mixing effect, flow to the stagnant zone would tend to be larger and the short circuiting effects smaller within a given reactor. The opposite effect is true at higher flow rates. The resulting data gave no indication that either short circuiting or stagnant zone effect was occurring in the vessel.

Compartment Inhomogeneity

The assumption was made at the outset that the compartments were, in fact, homogeneous, i.e., that a sample taken from one part of the compartment would yield the same analytical results as a sample from any other part. Sampling homogeneity was achieved.

However, an effect is observed due to the inviable portion of the cell compartment 2, and this effect is magnified when more radioactivity is present in the cells.

Data of the type collected in Run 15 (tagged HCO_3^-) would seem to be valuable in future work over the whole range of dilution rates. Such a study could conceivably quantitate the viability effect because the rate at which radioactivity moves across the viable-inviable boundary is a measure of the rate at which organisms become biochemically inviable.

Comments on Mathematical Model

The results would seem to bear out the initial assumption of linear first order differential equations to describe the kinetics of radioactivity movement within the system studies. More significantly, the transfer rates determined here are "overall" transfer rates in the sense that a specific biochemical reaction is not considered. This is not to say, however, that the technique, as applied here, is directly translatable to mixed microbiological populations. Due to the fact that the study was performed on a single organism metabolizing a single organic substrate, the number of biochemical reactions under consideration have been reduced to a minimum.

Variations of Yield Coefficient with Dilution Rate

The transfer rate of substrate carbon to cells (ρ_{12}), (Figure (5-6)), is observed to decrease with increasing dilution rate and decreasing generation time. The cells to inorganic carbon transfer rate, (ρ_{23}), (Figure (5-7)), is similarly observed to decrease with increasing dilution rate. As shown in Table (5-1) these variations coincide with decreasing total numbers of cells maintained in the chemostat and a decreasing carbon per cell ratio.

The cells to substrate carbon transfer rate, (ρ_{21}), (Figure (5-5)) is seen to reach a minimum value near dilution rate D = 0.5 hr^{-1}. The transfer rate of inorganic carbon to cells (ρ_{32}), (Figure (5-8)) reaches a maximum value near the same point. This minimum and maximum are observed to occur coincidentally with the maximum observed efficient yield coefficient. These variations in transfer rates ρ_{21} and ρ_{23} would appear to assume major significance in the interpretation of existing kinetic theory.

The effective yield coefficient (Y) in mg cell carbon per mg carbon utilized, in terms of carbon transfer rates, can be developed directly from Equation (3-5) and is given by

$$Y = 1 \frac{-\rho_{23} - \rho_{32}}{\rho_{12} - \rho_{21}} \qquad (5\text{-}10)$$

Using this relationship and the values of transfer rates obtained in this study, the yield coefficient was calculated. The resulting values are tabulated in Table (5-2). The variation in yield with dilution rate is illustrated in Figure (5-9). The decrease in effective yield coefficient at both high and low dilution rates is seen to be in the order of 10 percent.

The yield coefficient relationship with D is a very shallow curve with a maximum somewhere around D = 0.5 hrs^{-1} or 500 ml/hr flow rate. This relationship has previously been described as a constant (Herbert$^{(22)}$) and as a straight line variation with

TABLE 5-1

Carbon per Cell Ratio

Dilution Rate D_{-1} (hr^{-1})	Total Count (#/ml)	Carbon in Cells S_2 (mg)	Carbon per Cell (mg)
0.42	4.3×10^9	183	42×10^{-9}
0.50	2.8×10^9	151	56×10^{-9}
0.54	3.1×10^9	81	26×10^{-9}
0.60	4.2×10^9	45	11×10^{-9}

Inorganic Carbon Re-incorporation per Unit of Carbon Associated with the Active Mass

Dilution Rate (hr^{-1})	Transfer Rate ρ_{32} (mg/hr)	Carbon Associated with M_a (mg)	Ratio $\rho_{32}/(S_2)$ (hr^{-1})
0.42	6.1	102.5	0.060
0.50	9.1	87.9	0.104
0.51	7.0	82.9	0.084
0.54	7.8	45.2	0.172
0.59	2.1	25.1	0.084

TABLE 5-2

Effective Yield Coefficients

Run No.	Flow Rate Q (ml/hr)	Loading ρ_{61} (mgC/hr)	Dilution Factor D (hr^{-1})	Yield Coefficient	
				$\frac{mgC}{mgC}$	$\frac{mg\ cell}{mg\ COD}$
11	422	165	0.42	0.465	0.32
12	501	200	0.50	0.510	0.35
15	510	203	0.51	0.506	0.34
13	540	222	0.54	0.494	0.336
10	590	236	0.59	0.465	0.32

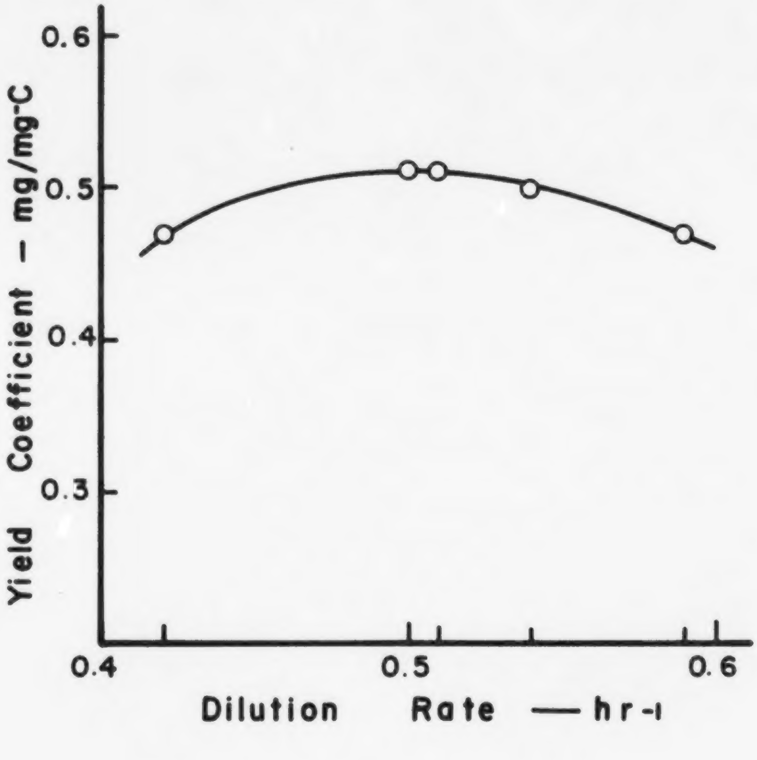

FIGURE 5-9 EFFECTIVE YIELD COEFFICIENT PSEUDOMONAS FLUORESCENS UTILIZING GLUTAMIC ACID

dilution rate in Chapter III of this project. Other workers have observed a maximum(53)(58). Maxon and Johnson(32) have reported on changes in yield coefficient with dilution rate. In one case they showed a maximum yield at about 0.5 hrs^{-1} dilution rate.

Literature values for observed yield coefficients on various substrates and organisms appear in Table 5-3. It should be noted that some studies observed the dry weight of organisms and others observed difference between influent and effluent substrate.

Herbert, et al.(22) noted that the yield coefficient decreased with increasing dilution rate and commented that his theoretical treatment had assumed that the yield coefficient was independent of growth rate and that this assumption was probably not valid. Table (5-3) includes some of Herbert's data for Aerobactic cloacae growing on glycerol; the yield was determined by measurement of bacterial dry weight and glycerol utilized in his chemostat. These data can also be interpreted as exhibiting a maximum yield near $D = 0.575\ hr^{-1}$.

Rosenberger and Elsden(58) report yield values for Streptococcus faecalis on glucose under conditions of tryptophan limitation in the continuous culture. Their study was not directed toward a mathematical description of the kinetics and they indicate that "the yield constant ... progressively fell with increasing supplies of glucose ...". It can be seen that these data may also be interpreted as exhibiting a maximum value near $D = 0.49\ hr^{-1}$.

Postgate and Hunter(53) have worked with Aerobactic aerogenes in the continuous culture under various nutrient limiting conditions,

TABLE 5-3

Reported Values for Observed Yield Coefficients

Reference	Growth Conditions	Dilution Factor (hr^{-1})	Yield
			$\frac{\text{gms bacteria}}{\text{gm glycerol}}$
(20)	Continuous	0.36	0.52
		0.50	0.53
		0.575	0.54
		0.70	0.50
		0.72	0.43
			$\frac{\text{cell dry wt.}}{\text{unit glucose}}$
(69)	Continuous	0.265	0.12
		0.305	0.16
		0.49	0.218
		0.61	0.215
		0.716	0.216
			$\frac{\text{mg dry wt.}}{\text{mg limiting nutrient}}$
(70)	Continuous (C-limiting)	0.25	1.38
		0.5	1.55
		0.72	1.17
(70)	Continuous (S-limiting)	0.12	220
		0.26	240
		0.41	200

at least two of which (carbon and sulfur) exhibit maximum yields of dry cells per mg of the limiting nutrient at intermediate dilution rates (see Table 5-3).

In Chapter III of this report the yield was measured as the difference between COD of the total chemostat contents and filtrate converted to cell weight divided by the difference between influent and filtrate COD. These data were obtained for Pseudomonas fluorescens growing on glutamic acid in the same chemostat apparatus used in this study. Because of the scatter of the COD data, it was grouped into intervals of dilution rate (e.g., 0.1 - 0.3; 0.2 - 0.4; etc.). If the individual values rather than the grouped data are plotted, however, data, as shown in Figure (5-10), can also be interpreted as exhibiting a maximum yield.

Maxon and Johnson(32) studied the growth of Baker's yeast on carbohydrate and proteinaceous carbon sources. Incidental to the aim of their study, they observed that in continuous culture, Baker's yeast metabolizing glucose exhibited a maximum effective yield at the maximum possible dilution rate prior to washout. This result is similar to the conclusion presented in Chapter III of this study. When metabolizing protein derivatives, however, they found a maximum yield in the mid-range of dilution rate which supports the findings presented herein. They felt that the different yield patterns were a function of the substrate chemical structure. Since the substrate used herein was glucose, this research cannot support their findings. It is, however, of interest that other researchers have observed a maximum yield at other than the maximum growth rate.

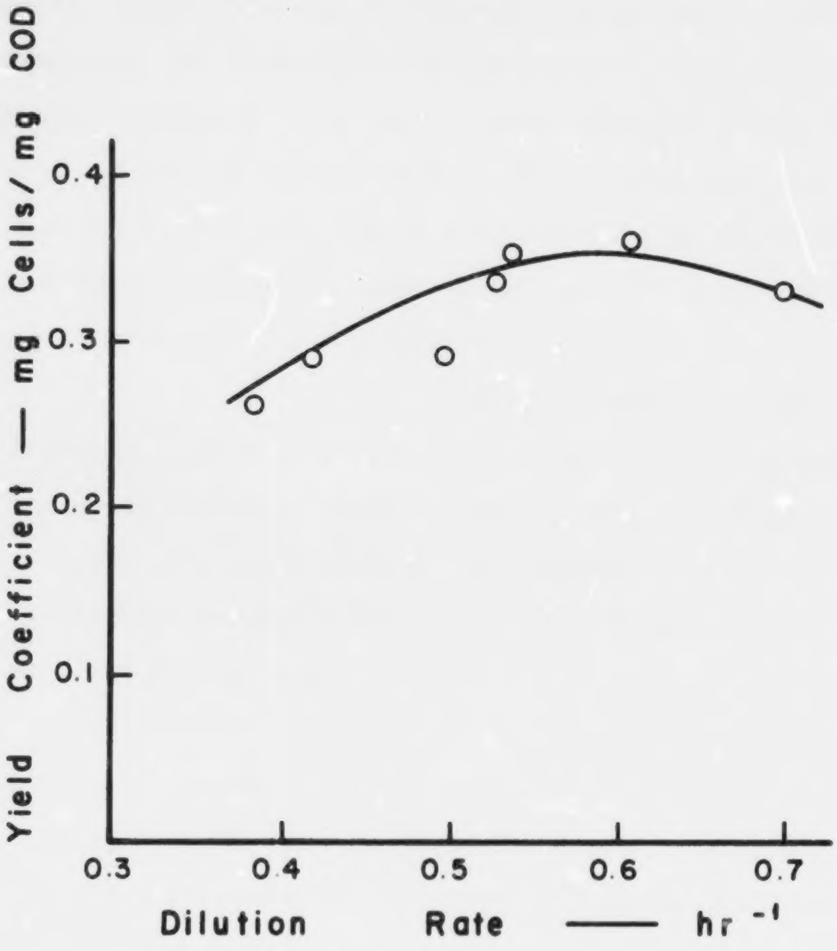

FIGURE 5-10 EFFECTIVE YIELD COEFFICIENT - RUN 2 PSEUDOMONAS FLUORESCENS UTILIZING GLUTAMIC ACID

It is significant to note at this point that while the observed decrease in the effective yield coefficient is in the order of 10 percent at $D = 0.5\ hr^{-1}$, the corresponding increase in ρ_{21}, the cells to substrate carbon transfer rate, is 50 percent at $D = 0.42\ hr^{-1}$, and almost 200 percent at $D = 0.59\ hr^{-1}$. The transfer rate measurements and yield coefficient measurements together would, therefore, seem to comprise a more informative parameter of the kinetics of the continuous culture. It is possible that variations in the yield coefficient have been overlooked in the past because the evidently small variations have been attributed either to experimental error or to partial failure of mathematical models proposed. It is impossible, however, to overlook the magnitude of the variations in ρ_{21} as observed in this study. It should be noted that, considering the standard deviation values, minimum values exhibited for ρ_{21} would exist even if yield coefficient was interpretated as a straight line.

The interpretation of the maximum value observed for ρ_{32} must necessarily be less decisive due to the large percentage standard deviation associated with it. In this regard, however, the results of Run 15 in which compartment 3 was the initially tagged compartment, indicate that the values obtained for ρ_{32} are in the range indicated. The standard deviation of ρ_{32} is proportionally less when determined by the technique used in Run 15 (see Table C-11).

This research definitely indicates that the effective yield (Y) exhibits a maximum value in the mid-range of dilution rates.

As described above, there are data in the literature which can be interpreted as supporting these results. It is necessary to consider the proposed mathematical models for growth kinetics of bacteria to evaluate what terms might cause the observed phenomenon.

It was observed that the contribution of gross substrate from the cells is a significant quantity. The value of ρ_{21}, as shown in Figure (5-5), ranged from 20 to over 60 mg/hr. This was a significant percent of the rate of substrate utilization (ρ_{12}) which had a maximum value of 200 mg/hr. It is evident that pq in Equation (3-2a) cannot be ignored. Therefore, Equations (3-1b) and (3-2a) should be considered when deriving an expression for Y in terms of the proposed coefficient. Following the procedure used in deriving the yield formula in Equation (3-11) but using these Equations

$$Y = \frac{1}{\frac{1}{2} + \frac{K_1}{D} + \frac{K_2}{D}\left(\frac{1}{Z} - pq\right)} \tag{5-11}$$

Expressing this equation as a straight line

$$\frac{1}{Y} = \frac{1}{2} + \left[K_1 + K_2 \left(\frac{1}{Z} - pq\right)\right] \frac{1}{D} \tag{5-12}$$

This equation will be a straight line only if the various coefficients are constants with the only variables being Y and D.

It is unlikely that the true yield coefficient (Z) is a variable for a given organism growing on a single substrate, since it is a measure of the true ability of the organism to synthesize the substrate. Gunsalus and Shuster$^{(19)}$ have discussed the variations in ATP yield with metabolic pathway. If it can be assumed that the Z is a function of ATP yield as suggested in Chapter III, then, for a single organism and substrate, it can be assumed that a fixed set of metabolic pathways is followed and Z will be a constant. It was shown previously that Z varies with the organism and substrate used.

The energy metabolism coefficient (K_1) represents uptake of substrate for the energy for minimal maintenance requirements, e.g., flagellar motion and energy for active transport across the cell membrane. It is conceivable that K_1 could vary depending upon the growth rate of the organisms (or D) but these variations - from the definition of energy metabolism - would be included in the transfer rate of carbon from substrate to cells (ρ_{12}) illustrated on Figure (5-6), and not in the transfer rate ρ_{21}.

The terms in Equation (5-12) which remain for consideration are

p = fraction of cells which die and lyse.
q = fraction of exogenous substrate released per unit of cells lysed.
K_2 = death coefficient.

The observed variation of ρ_{21} with D (Figure (5-5)) indicates that more carbon per unit time is transferred back to compartment 1 at values of $D > 0.5$ and $D < 0.5$ than at $D = 0.5$. When $D < 0.5$, the growth rate is low (generation time is high) and concentration of exogenous substrate is low. When $D > 0.5$, the growth rate is higher (generation time is low) in order to maintain the culture at high flow rates, and the concentration of exogenous substrate is relatively high. These observations must be kept in mind during the following discussion.

Two contributive sources of exogenous substrate other than that which enters in the influent are

1. from lysed cells - the effect of which is included in p and q.
2. from whole cells - whether it be release of biochemical intermediated from biochemically active cells or osmotic release from cells which have ceased to be biochemically active - the overall effect of which is included in K_2 itself.

A number of investigators have observed variations in autolytic activity with both substrate concentration and growth rate(40(64)(65)(66).

Shockman, et al.(65) showed that rapid lysis or Streptococcus faecalis occurred in either phosphate buffer or NaCl providing the cells were either depleted of a nutrient essential for synthesis of cell wall material or the cells were taken from a culture growing in the exponential or log growth phase. When cells were depleted of an amino acid which was not a major component of cell wall material, they observe little or no lysis. Specifically, glutamic acid is a major constituent of the cell wall of S. faecalis and its depletion in the medium led to lysis(65)(66).

Shockman, et al.(64) made the observation that S. faecalis lysine - limited cells behaved similarly to exponentially growing cells when transferred to a lysine free medium and their conclusion was that in the absence of continued renewal, the amount of cell wall possessed by the organisms was inadequate for their maintenance.

They also made the statement that the generally greater sensitivity of physiologically young cells to, e.g., cold shock, ultraviolet irradiation, etc., may be attributed, at least in part, to differences in amount of cell wall.

Mitchell and Moyle(40) also studied the effect of growth phase on autolytic tendency. Staphylococcus aureus growing on 1.2 M - sucrose (acetic buffered) were subjected to 1.2 M NaCl solutions at various growth stages and turbidity measurements were made with time. The ratio of turbidity values obtained on incubation without NaCl to that obtained with NaCl were used as a measure of osmotic fragility. The time required for 50 percent of the organisms to become osmotically fragile was the parameter. The time for development of 50 percent osmotic fragility for organisms from the exponential phase of growth was observed at 25 minutes. Electron photomicrographic observations included in the study indicate that the observed effects were, in fact, due to breaking of cells. The time for 50 percent of cells to become osmotifragile increases rapidly in the area of non-nutrient limiting circumstances.

The above discussion would suggest that there is a slowing in the rate of synthesis of cell wall material at higher growth rates. This conclusion appears to be borne out by the results presented in Table (5-1) which indicate a decrease in the amount of carbon per cell with increasing dilution rate. It could also be said, however, that the whole cell merely decreases in size at higher D. Table (5-1) also shows values of re-incorporation of organic carbon per unit of carbon associated with the active mass.

SUMMARY

There are at least two contributing factors to the observed variations in ρ_{21} and subsequently the effective yield coefficient. These are a) substrate released as a result of autolytic activity and b) biochemical end products released from active cells as a result of partial metabolism of the substrate. Further complicating the considerations is the observation that lysis is probably occurring at low dilution rates and has the possibility of occurring at high dilution rates. The relative contributions and magnitudes of p, q and K_2 at different dilution rates would appear unresolvable until further work is done which can differentiate between autolytic versus biochemically released substrate.

Even though this study was performed with a pure culture on a single organic substrate, there would seem to be a number of areas of engineering significantly affected by the results.

The objective of the activated sludge process is to remove organic waste material from the water carrier. If the variations of Y vs. D found here hold true, for activated sludge, this end can be accomplished at any of the lower dilution rates. Even though at low dilution rates the microorganisms may put more potential substrate back into the solution in the form of primary metabolic end products, they also take substrate up much faster to support a large population. At these operating conditions, substrate is removed as fast as it enters the reactor. This is, of course, the basis of operation of the extended aeration type processes. These results would indicate that the effective yield at the high dilution rates would have a decreasing trend. It is possible that this decrease in effective yield could be used to advantage in activated sludge processes using high process loadings and short detention periods. Further research is needed in this area.

As industrial applications requiring maximum production of organisms, for example, yeast, it would seem that the technique described herein would be used to determine the process loading for optimum production. The yield could be affected by perhaps 10 percent for any substrate loading.

From the results of this study, it must be concluded that the maximum cell yield does not necessarily correspond to the maximum yield of biochemical end products. This conclusion may have significance with respect to the production of antibiotics, for example, where it is necessary to know the growth conditions for maximum production for biochemical end products(3)(51)(55).

In the past, these operations have been based on results obtained from batch culture kinetics. The use of the continuous culture kinetics and transfer rate techniques presented here may find considerable application in the fermentation industry.

CHAPTER VI - CONCLUSIONS

As this research project has proceeded various investigations have been conducted. The sequence of and approach used in these investigations was a result of the findings of previous investigations. Following this procedure of progressive research, considerable progress has been made toward a better understanding of the kinetics of bacterial growth. Some of the more significant conclusions which can be drawn from these investigations are presented here.

1. Because of the inherent variations in the mixed culture population of activated sludge, it is not a satisfactory system for use in investigation aimed at improving deterministic mathematical models of substrate removal or sludge synthesis.

2. The effective yield coefficient is not a constant but varies, not only with the organism-substrate system, but with the process loading. *Pseudomonas fluorescens* grown on glutamic acid exhibited a maximum yield at a mid-range process loading.

3. Internal transfer rates, as measured by the radioisotope technique described herein, indicate that the contribution of exogenous substrate from metabolizing organisms is significant and should not be neglected in the development of culture kinetic models.

4. *Pseudomonas fluorescens* growing on glutamic acid exhibits a minimum production of exogenous substrate at the dilution rate corresponding to the maximum observed effective yield coefficient. Further, the variation in exogenous substrate transfer rate was many fold the variation in effective yield coefficient.

5. The true yield coefficient is not necessarily proportionate to the change in the free energy of oxidation of the substrate utilized.

6. A mixed culture and/or complex media gives a higher effective yield than a pure culture on a simple media.

7. When a gross measure of substrate concentration is used, any formula for synthesis rate should be modified to consider the residual organic material arising from the culture's activity.

8. The form of the proposed mathematical models for viable cell accumulation (Equation (3-1a)) and for gross substrate removal (Equation (3-2b)) was supported by this research. However, the delineation of the coefficients in terms of process loading waits further changes in analytical approach to the problems encountered in their evaluation.

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APPENDIX A

NOMENCLATURE

Nomenclature

A system nomenclature has been developed which, it is hoped, will suffice not only for this work but for current and future mathematical descriptions of the kinetics of bacterial growth. The complete set of symbols is included here although not all of them were used in this report.

A_A = Active mass accumulation (wt./vol.-time)

A, B = A constant in the relationship proposed between substrate concentration and growth rate (wt./vol.)

A_T = Total mass accumulation (wt./vol.-time)

A_V = Volatile mass accumulation (wt./vol.-time)

B = Yield coefficient-accumulation of total mass resulting from substrate removed for synthesis (gross measure)

C = Yield coefficient-synthesis of active mass resulting from substrate removed for synthesis (gross measure)

D = Dilution rate in the reaction vessel ($time^{-1}$)

E = Efficiency of substrate removal

F_{AB} = Total substrate applied as BOD (wt./time)

F_R = Total substrate removed (gross measure) (wt./time)

F_{R}' = Substrate removed for synthesis only (gross measure) (wt./time)

F_{RB} = Total substrate removed as BOD (wt./time)

f = A constant in previously proposed relationships between substrate concentration and growth rate (wt./vol.)

J = Fraction of active mass that, as a result of auto-digestion, becomes inert volatile solids

K_{ei} = Rate of release of combined end product and biochemical intermediates from cells back into available substrate pool (wt. material/wt. cells-time)

K_s = Substrate concentration that will yield one-half the maximum growth rate (wt./vol.)

K_1 = General substrate removal rate for energy metabolism by active mass ($time^{-1}$)

K_1' = Specific substrate removal rate for energy metabolism by active mass ($time^{-1}$)

K_2 = Heterogenous metabolism rate, i.e. endogenous metabolism rate plus autolysis rate (wt. cells/wt. cells-time)

K_2' = Heterogenous metabolism rate based upon M_T (wt. cells/wt. cells-time)

L = Volumetric process loading (wt./vol.-time)

L_r = Volumetric process removal (wt./vol.-time)

M or M_v = Concentration of total volatile mass in reaction vessel (wt./vol.)

M_a = Concentration of active mass in reaction vessel (wt./vol.)

M_T = Concentration of total mass in reaction vessel (wt./vol.)

p = Fraction of cells which die and lyse in the reaction vessel (wt./wt.)

P_A = Active mass production (wt./vol.-time)

P_T = Total mass production (wt./vol.-time)

P_v = Volatile mass production (wt./vol.-time)

q = Fraction of exogenous substrate released per unit of cells lysed (wt./wt.)

Q = Flow rate (vol./time)

R = True yield coefficient-Removal of specific influent substrate for synthesis only.
NOTE: It should be clear that R is the synthesis of active mass resulting from the substrate removed for synthesis and corrected for the presence of autolysis, biochemical intermediates and end product fractions (gross measure) in the mixed liquor.

S_c = Specific substrate concentration in the reaction vessel (wt./vol.)

S_c' = Total substrate concentration in the reaction vessel (wt./vol.)

S_1 = General substrate concentration in the incoming feed (wt./vol.)

S_1' = Specific substrate concentration in the incoming feed (wt./vol.)

S_{1B} = General substrate concentration in the incoming feed as BOD (wt./vol.)

t = Time

$T_d = \frac{1}{D} = \frac{V}{Q}$ = Detention time of the reaction vessel (time)

V = Volume of the reaction vessel (vol.)

x = A constant in previously proposed relationships between substrate concentration and growth rate (wt./vol.)

X = Yield coefficient-synthesis of active mass resulting from the removal of substrate (gross measure)

Y = Effective yield coefficient-accumulation of active mass resulting from the removal of substrate (gross measure)

Z = Yield coefficient-synthesis of active mass resulting from the substrate removed for synthesis (gross measure) and corrected for the presence of substrate made available through autolysis in the mixed liquor.

μ = True growth rate of active mass (wt. cells/wt. cells-time)

μ_e = Effective growth rate of active mass (wt. cells/wt. cells-time)

μ_e' = Effective growth rate of active mass based upon total mass (wt. cells/wt. cells-time)

μ_e'' = Effective growth rate of suspended volatile solids (wt. cells/wt. cells-time)

μ_m = Maximum growth rate of active mass (wt. cells/wt. cells-time)

APPENDIX B

GENERAL SYNTHESIS DATA

TABLE B-1

Industrial Waste Analysis

Source: Production and clean-up wastes mixed with cooling water and sanitary wastes from Orchard Hill Farms, Red Hook, New York, manufacturers of TV dinners and pot pies.

Date of Supply: February 18, 1964

Partial Analysis of Raw Wastes: *	pH	11
	COD	9,400 mg/l
	BOD	5,700 mg/l
	ABS	100 mg/l

*In order to use the waste as a media, it was diluted to 1,000 mg/l COD and the pH adjusted to 6.8 with sulfuric acid.

TABLE B-2

Continuous Culture Run 1
Analytical Results
Pseudomonas fluorescens Utilizing Glucose

Sample #	pH	COD mg/l		Dilution Rate 1/hr.
		Total	Dissolved	
8/27/3A	7.0	394	81	0.23
8/28/3A	6.7	353	80	0.23
9/6/3A	6.9	810	551	0.51
9/6/3B		765	597	0.52
9/10/3A	6.8	809	600	0.58
9/10/3B		795	515	0.58
9/11/3A	6.8	1,067	987	0.78
9/12/3A		1,020	970	0.78
9/13/3A		1,015	945	0.89
9/13/3B		1,000	930	0.89
9/13/3C		1,000	945	0.89
8/22/2A		469	91	0.37
8/23/2A		445	89	0.43
8/26/2A		434	77	0.09
8/27/2A		430	155	0.16
8/27/2B		381	95	0.16
8/28/2A		381	78	0.17
8/28/2B		363	114	0.17
8/29/2A		363	113	0.17

TABLE B-2

(continued)

Sample #	pH	COD mg/1 Total	COD mg/1 Dissolved	Dilution Rate 1/hr.
8/29/2B		341	120	0.33
9/4/2A		310	99	0.32
9/6/2A		407	106	0.34
9/6/2B		585	100	0.38
9/7/2A		615	112	0.38
9/10/2A		615	587	0.57
9/10/2B		805	590	0.57
9/10/2C		565	477	0.62
9/11/2A		889	891	0.80
9/12/2A		889	691	0.63
9/12/2B		785	690	0.62
9/13/2A		1,042	1,108	0.85
9/13/2B		841	1,095	0.88
9/13/2C		1,314	907	0.79

TABLE B-3

Continuous Culture Run 2
Analytical Results
Pseudomonas fluorescens Utilizing Glutamic Acid

Sample #	pH	COD mg/1 Total	COD mg/1 Dissolved	Dilution Rate 1/hr.
2/1/1A	7.1	915	758	0.60
2/1/2A	7.2	465	73	0.42
2/1/3A	7.0	750	521	0.53
2/1/4A		400	56	0.21
2/1/5A	6.8	835	815	0.59
2/1/6A	7.2	331	44	0.21
2/3/1A	7.1	325	755	0.30
2/3/2A	7.1	355	125	0.26
2/3/3A	7.2	386	23	0.24
2/3/4A		465	93	0.28
2/3/5A	7.3	289	34	0.10
2/3/6A	7.2	424	58	0.10
2/4/1A	7.0	802	944	0.82
2/4/2A	7.2	883	822	0.54
2/4/1B		807	787	0.71
2/4/2B		450	252	0.34
2/4/3B	7.1	991	915	0.74
2/4/4B		1,075	974	0.78
2/4/5B	7.0	549	143	0.28

TABLE B-3

(continued)

Sample #	pH	COD mg/l Total	COD mg/l Dissolved	Dilution Rate 1/hr.
2/4/6B	7.0	826	697	0.50
2/4/4A		595	345	0.38
2/4/5A	6.9	505	45	0.23
2/5/1A	7.0	429	26	0.18
2/5/2A		1,075	1,075	1.02
2/5/3A		955	658	0.78
2/5/4A		905	796	0.61
2/5/5A		561	45	0.26
2/5/6A	7.0	814	641	0.70
2/5/4B		1,000	1,000	1.00
2/22/4A		485	65	0.15
2/23/4A		445	25	0.20
2/24/4A		430	0	0.18
2/24/4B		345	25	0.18

TABLE B-4

Continuous Culture Run 3
Analytical Results
Pseudomonas fluorescens Utilizing Sodium Acetate

Sample #	pH	COD mg/l Total	COD mg/l Dissolved	Dilution Rate 1/hr.
2/14/1A	7.0	269	43	0.02
2/14/1B		367	43	0.12
2/15/1A	6.9	279	34	0.14
2/15/2A	7.0	367	34	0.18
2/15/3A		288	99	0.20
2/15/4A	7.2	230	14	0.06
2/15/5A	7.1	288	53	0.15
2/15/6A	6.8	237	66	0.17
2/16/1A		286	0	0.30
2/16/2A	7.0	846	720	0.82
2/16/3A		279	34	0.16
2/16/4A		337	34	0.26
2/10/5A		337	4	0.26
2/16/2A		337	34	0.18
2/17/1A	7.0	316	53	0.36
2/17/2A	7.0	770	720	0.82
2/17/3A	7.1	310	36	0.38
2/17/4A	6.7	711	490	0.66
2/17/5A	7.0	1,283	874	1.00

TABLE B-4

(continued)

Sample #	pH	COD mg/l		Dilution Rate 1/hr.
		Total	Dissolved	
2/17/6A	7.0	312	35	0.12
2/18/6A	7.0	311	15	0.26
2/18/1B	7.0	815	750	0.80
2/18/2B	7.0	217	66	0.14
2/18/4B	6.8	985	904	0.90
2/18/6B		761	721	0.76
2/13/5B	7.2			0.11

TABLE B-5

Continuous Culture Run 4
Analytical Results
Escherichia coli B Utilizing Sodium Acetate

Sample #	pH	COD mg/l Total	COD mg/l Dissolved	Dilution Rate 1/hr.
1/2/1A	6.8	430	77	0.07
1/7/1A				0.12
1/8/1A	6.7	405		0.26
1/9/1A		539	173	0.32
1/10/1A				0.45
1/11/1A	6.5	1,035	995	0.76
1/13/1A	6.5	1,305	935	0.67
1/2/2A		421	48	0.10
1/3/2A		386	64	0.06
1/6/2A		430	120	0.01
1/7/2A				0.40
1/10/2B		1,025	975	0.86
1/8/2A				0.42
1/11/2A		816	760	0.56
1/13/2A	6.9	393	116	0.01
1/2/3A				0.60
1/3/3A		466	153	0.34
1/4/3A		491	153	0.34
1/6/3A		337	93	0.01

TABLE B-5

(continued)

Sample #	pH	COD mg/l		Dilution Rate 1/hr.
		Total	Dissolved	
1/7/3A		489	96	0.32
1/8/3A	6.0	995	995	0.65
1/9/3A	6.1	1,045		0.69
1/9/3B		975	995	0.69
1/10/3A	6.8	538	112	0.41
1/11/3B	6.7	419		0.54
1/13/3A				0.55
1/3/4A			64	0.09
1/4/4A		441	101	0.09
1/6/4A		375	55	0.01
1/9/4A		685	525	0.57
1/4/5A		489	93	0.17
1/6/5A		372	91	0.17
1/8/5B		735	675	0.58
1/9/5A		725	485	0.53
1/10/5A	6.9	450	84	0.12
1/3/5A				0.17
1/3/5B		465	106	0.17
1/2/6A		325	106	0.05
1/3/6A		451	56	0.12
1/4/6A		475	53	0.11

TABLE B-5

(continued)

Sample #	pH	COD mg/l Total	COD mg/l Dissolved	Dilution Rate 1/hr.
1/6/6A		440	111	0.10
1/7/6A				0.35
1/8/6A	6.9	417	132	0.36
1/8/6B		455	165	0.36
1/9/6A		425	185	0.38
1/9/6B		555	195	0.38
1/10/6B		935	935	0.92
1/11/6A	6.3	1,025	842	0.62
1/13/6A		241	108	0.06
1/13/6B		474	106	0.12

TABLE B-6

Continuous Culture Run 5
Analytical Results
Escherichia coli B Utilizing Glutamic Acid

Sample #	pH	COD mg/l		Dilution Rate 1/hr.
		Total	Dissolved	
1/19/1A		401	191	0.67
1/20/1A	6.9	272	66	0.06
1/20/1B		377		0.08
1/21/1A		705	569	0.94
1/21/1B		1,000	960	1.39
1/18/2A		460	53	0.29
1/20/2A	6.7	743	167	0.73
1/18/2B		436	43	0.30
1/20/2B		706	439	0.75
1/21/2A		496	58	0.47
1/21/2B		652	223	0.53
1/22/2A		876	960	1.28
1/18/3A		835	835	1.11
1/18/3B		877	783	1.11
1/20/3A	6.4	680	310	0.85
1/20/3B		757	674	0.90
1/21/3A		350	79	0.28
1/21/3B		450	78	0.26
1/22/3A	6.8	834	754	1.10

TABLE B-6

(continued)

Sample #	pH	COD mg/1 Total	COD mg/1 Dissolved	Dilution Rate 1/hr.
1/18/4A		426	34	0.36
1/18/4B		416	52	0.36
1/20/4A	6.6	407	60	0.38
1/20/4B		397	78	0.40
1/21/4A		736	506	0.90
1/22/4A	6.8	930	870	1.58
1/22/4B		930	940	1.58
1/18/5A		770	509	0.85
1/18/5B		601	181	0.55
1/20/5A				0.39
1/20/5B	6.8	425	67	0.39
1/21/5A		587	230	0.75
1/22/5A	6.8	430	72	0.31
1/22/5B		409	58	0.32
1/18/6A		366	43	0.05
1/18/6B		465	63	0.03
1/20/6A				0.03
1/20/6B	6.8	401	90	0.12
1/21/6A		394	100	0.16
1/22/6A	7.0	965	853	1.01
1/22/6B		1,000	990	1.50
1/23/6C		1,010	990	1.55

TABLE B-7

Continuous Culture
Analytical Results
Miscellaneous Experiments

Sample #	pH	COD mg/1 Total	COD mg/1 Dissolved	Dilution Rate 1/hr.
Bacillus cereus Utilizing Glutamic Acid				
2/22/1A		460	84	0.20
2/23/1A		385	16	0.21
2/24/1A		369		0.20
2/24/1B		445	35	0.15
Alcaligenes faecalis Utilizing Glutamic Acid				
2/25/2A		559	163	0.09
2/25/2B		491	177	0.10
2/26/2A		511	177	0.15
Alcaligenes faecalis and Pseudomonas fluorescens Utilizing Glutamic Acid				
2/27/2A		521	197	0.17
2/28/2A		485	175	0.18
2/28/2B		485	75	0.17
2/29/2A		465	75	0.16
2/29/2B		595	135	0.16
3/1/2A		549	143	0.17

TABLE B-7

(continued)

Sample #	pH	COD mg/1 Total	COD mg/1 Dissolved	Dilution Rate 1/hr.
Bacillus cereus, Alcaligenes faecalis and Pseudomonas fluorescens Utilizing Glutamic Acid				
2/25/3A		531	36	0.07
2/25/3B		525	25	0.07
2/26/3A		480	6	0.15
2/26/3B		436	53	0.15
2/27/3A		455	65	0.20
2/28/3A		455	55	0.20
2/28/3B		455	73	0.21
2/29/3A				0.22
2/29/3B		456	73	0.22
Pseudomonas fluorescens Utilizing Synthetic Sewage				
2/28/5A		317	69	0.13
2/28/5B		462	114	0.13
2/29/5A		577	46	0.14
2/29/5B		435	35	0.15
3/1/5A		565	15	0.15
3/2/5A		565	91	0.14
3/3/5A		587	46	0.14

TABLE B-7

(Continued)

Sample #	pH	COD mg/l Total	COD mg/l Dissolved	Dilution Rate 1/hr
Pseudomonas fluorescens Utilizing Industrial Wastes				
2/28/1A		430	180	0.13
2/28/1B		460	50	0.13
2/29/1A				0.14
2/29/1B		470	180	0.13
3/1/1A		485	178	0.13
3/2/1A		455	182	0.12
3/3/1A		449	167	0.12
Pseudomonas fluorescens Utilizing Mixed Monomers				
2/28/6A		381	143	0.13
2/28/6B		401	183	0.13
2/29/6A		414	149	0.14
2/29/6B		405	165	0.13
3/1/6A		515	85	0.13
3/2/6A		462	86	0.12
3/3/6A		565	97	0.12

TABLE B-8

Calculated Effective Yield Coefficients

Dilution Interval	Rate Average	mg/l Substrate COD Utilized	mg/l Cells Formed	Effective Yield Coefficient mg cells/ mg Substrate COD
Run 1 *Pseudomonas fluorescens* Utilizing Glucose				
0.0-0.2	0.15	1,005	195	0.19
0.1-0.3	0.18	1,008	190	0.19
0.2-0.4	0.32	1,011	228	0.23
0.3-0.5	0.36	1,008	248	0.25
0.4-0.6	0.54	606	147	0.24
0.5-0.7	0.58	521	116	0.22
0.6-0.8	0.70	323	104	0.32
0.7-0.9	0.84	124	30	0.24
Run 2 *Pseudomonas fluorescens* Utilizing Glutamic Acid				
0.0-0.2	0.16	947	252	0.27
0.1-0.3	0.20	923	258	0.27
0.2-0.4	0.27	877	341	0.26
0.3-0.5	0.36	797	199	0.25
0.4-0.6	0.53	369	112	0.30
0.5-0.7	0.58	262	86	0.33
0.6-0.8	0.72	188	88	0.47
Run 3 *Pseudomonas fluorescens* Utilizing Sodium Acetate				
0.0-0.2	0.13	992	165	0.17
0.1-0.3	0.19	993	176	0.18
0.2-0.4	0.30	1,008	194	0.19
0.3-0.5	0.35	998	178	0.18
0.4-0.6	*	*	*	*
0.5-0.7	*	*	*	*
0.6-0.8	0.74	384	73	0.19
0.7-0.9	0.82	294	60	0.20

TABLE B-8

(Continued)

Dilution Interval	Rate Average	mg/1 COD	Substrate Utilized	mg/1 Cells Formed	Effective Yield Coefficient mg Cells/ mg Substrate COD
Run 4 *Escherichia coli* B Utilizing Sodium Acetate					
0.0-0.2	0.09		949	220	0.23
0.1-0.3	0.14		953	245	0.25
0.2-0.4	0.34		897	226	0.25
0.3-0.5	0.36		884	227	0.26
0.4-0.6	0.52		543	145	0.27
0.5-0.7	0.62		215	56	0.27
Run 5 *Escherichia coli* B Utilizing Glutamic Acid					
0.0-0.2	0.08		896	209	0.23
0.1-0.3	0.24		896	227	0.25
0.2-0.4	0.33		908	241	0.26
0.3-0.5	0.36		912	320	0.35
0.4-0.6	0.52		814	292	0.36
0.5-0.7	0.58		770	240	0.31
0.6-0.8	0.73		711	239	0.34
0.7-0.9	0.82		563	208	0.37

*Insufficient data collected.

APPENDIX C

CARBON TRANSFER DATA

TABLE C-1

Compartment 1

Carbon Measurements - Calculated Error

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation from Mean (mg)	Percent Deviation* (%)
11	0.5	0.0264	-0.0022	3.8
	1.5	0.0286	±0.0000	3.4
	2.0	0.0308	±0.0022	3.2

s = 0.0022, p = 0.001, E_1 = 14.3 ± 0.7 mg

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation from Mean (mg)	Percent Deviation* (%)
12	0.083	0.162	-0.006	2.0
	0.5	0.167	-0.001	2.0
	1.0	0.166	-0.002	2.0
	1.5	0.167	-0.001	2.0
	2.0	0.176	±0.008	1.9
	2.5	0.172	±0.004	1.9

s = 0.005, p = 0.003, E_1 = 84 ± 2.2 mg

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation from Mean (mg)	Percent Deviation* (%)
15	0.083	0.229	±0.002	0.9
	0.5	0.221	-0.006	0.9
	1.0	0.230	±0.003	0.9
	1.5	0.226	-0.001	0.9
	2.0	0.228	±0.001	0.9

s = 0.0035, p = 0.002, E_1 = 83.2 ± 0.9 mg

TABLE C-1 (Continued)

Compartment 1

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation from Mean (mg)	Percent Deviation* (%)
13	0.083	0.463		0.43
	0.5	0.454		0.44
	1.0	0.443		0.45
	1.5	0.433		0.46
	2.0	0.430		0.46
	2.5	0.400		0.50

use $p = 0.002$, $E_1 = 200 \pm 1.5$ mg

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation from Mean (mg)	Percent Deviation* (%)
10	0.083	0.277	±0.004	2.4
	0.5	0.268	-0.005	2.4
	1.0	0.283	±0.010	2.3
	2.0	0.261	-0.012	2.5
	2.5	0.274	±0.001	2.4

$s = 0.0085$, $p = 0.006$, $E_1 = 273 \pm 9$ mg

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*calculated as the ratio of the absolute value of the probable error to the measured carbon value; $p = 0.674\,\sigma$ (normal)

TABLE C-2

Compartment 2

Carbon Measurements -- Calculated Error

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation from Mean (mg)	Percent Deviation* (%)
11	0.083	0.832	±0.000	.7
	1.0	0.817	-0.015	.7
	1.5	0.832	±0.000	.7
	2.0	0.840	±0.008	.7
	2.5	0.840	±0.008	.7

s = 0.009, p = 0.006, E_2 = 183 ± 1.5 mg

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation from Mean (mg)	Percent Deviation* (%)
12	0.5	0.778	-0.008	1.0
	1.0	0.802	±0.016	1.0
	1.5	0.795	±0.009	1.0
	2.0	0.778	-0.008	1.0
	2.5	0.778	-0.008	1.0

s = 0.012, p = 0.008, E_2 = 157 ± 2.4 mg

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation from Mean (mg)	Percent Deviation* (%)
15	0.083	0.748	±0.006	1.2
	0.5	0.721	-0.021	1.2
	1.0	0.734	-0.008	1.2
	1.5	0.755	±0.013	1.2
	2.0	0.751	±0.009	1.2

s = 0.014, p = 0.009, E_2 = 148 ± 3 mg

TABLE C-2 (Continued)

Compartment 2

Run No.	Elapsed Time (hrs)	Measured Carbon (mg)	Deviation From Mean (mg)	Percent Deviation* (%)
13	0.083	0.405		1.5
	0.5	0.503		1.5
	1.0	0.443		1.4
	1.5	0.468		1.3
	2.0	0.500		1.2
	2.5	0.540		1.1

use $p = 0.006$, $E_2 = 105 \pm 1.5$ mg

10	0.5	0.211	-0.013	4.5
	1.0	0.248	+0.024	4.5
	1.5	0.220	-0.004	4.5
	2.0	0.227	+0.003	4.5
	2.5	0.212	-0.012	4.5

$s = 0.015$, $p = 0.010$, $E_2 = 45 \pm 3$ mg

*calculated as the ratio of the absolute value of the probable error to the measured carbon value; $p = 0.674\,\sigma$ (normal)

TABLE C-3

Compartment 1

Specific Activity and Radioactivity Values with Associated Probable Error

Run No.	Elapsed Time (hr)	Specific Activity $a_1 \pm p_{a_1}$ (nc/mg)	Radioactivity $R_1 \pm p_{R_1}$ (nc)
11	1.5	43 ± 1.6	616 ± 38
	2.0	32 ± 1.2	450 ± 28
	2.5	26 ± 1.8	369 ± 31
12	0.083	560 ± 12.0	47,000 ± 1300
	0.5	301 ± 6.0	25,300 ± 680
	1.0	107 ± 2.0	9,000 ± 240
	1.5	47 ± 1.0	3,950 ± 110
	2.0	22.4 ± 0.5	1,880 ± 50
	2.5	11.8 ± 0.2	990 ± 30
13	0.083	268 ± 2.0	53,700 ± 590
	0.5	175 ± 1.4	35,000 ± 380
	1.0	110 ± 0.9	22,000 ± 240
	1.5	74.4 ± 0.6	14,900 ± 160
	2.0	50.1 ± 0.4	10,000 ± 110
	2.5	33.1 ± 0.3	6,600 ± 70
15	0.083	1.05	
	0.5	0.95	
	1.0	0.65	
	1.5	0.40	
	2.0	0.39	
10	0.083	301 ± 5.0	82,000 ± 2300
	0.5	176 ± 3.0	48,000 ± 1600
	1.0	120 ± 2.0	33,000 ± 1100
	1.5	87.5 ± 1.6	24,900 ± 800
	2.0	63.0 ± 1.0	17,200 ± 590
	2.5	43.3 ± 0.7	11,800 ± 400

TABLE C-4

Compartment 2

Specific Activity and Radioactivity Values with Associated Probable Error

Run No.	Elapsed Time (hr)	Specific Activity $a_2 \pm p_{a_2}$ (nc/mg)	Radioactivity $R_2 \pm p_{a_2}$ (nc)
11	0.083	211 ± 2.0	21,600 ± 260
	0.5	263 ± 2.5	27,000 ± 320
	1.0	235 ± 2.3	24,000 ± 290
	1.5	221 ± 2	22,700 ± 270
	2.0	162.5 ± 1.6	16,600 ± 200
	2.5	134 ± 1.2	13,900 ± 170
12	0.083	75 ± 0.9	6,400 ± 120
	0.5	185 ± 2.1	16,300 ± 300
	1.0	211 ± 2.5	18,500 ± 350
	1.5	197 ± 2.3	17,300 ± 330
	2.0	169 ± 2.3	14,800 ± 280
	2.5	125.2 ± 1.4	11,000 ± 200
15	0.083	7.2 ± 0.1	590 ± 14
	0.5	22.1 ± 0.3	1,800 ± 43
	1.0	25.2 ± 0.3	2,090 ± 50
	1.5	22.7 ± 0.3	1,880 ± 45
	2.0	20.4 ± 0.3	1,690 ± 40
13	0.083	43.4 ± 0.7	2,550 ± 50
	0.5	86.8 ± 1.4	5,100 ± 100
	1.0	110 ± 1.6	6,450 ± 130
	1.5	118 ± 1.6	6,950 ± 150
	2.0	109 ± 1.4	6,380 ± 120
	2.5	97.1 ± 1.2	5,700 ± 100
10	0.083	51.2 ± 1.6	1,260 ± 90
	0.5	84.6 ± 2.7	2,130 ± 150
	1.0	111 ± 3.4	2,790 ± 200
	1.5	122 ± 3.8	3,080 ± 220
	2.0	116 ± 3.6	2,900 ± 200
	2.5	110 ± 3.4	2,760 ± 190

TABLE C-5

Compartment 3

Specific Activity Values with Associated Probable Error

Run No.	Elapsed Time (hr)	Rate of Change of Activity dR_5/dt (nc/hr)	Specific Activity $a_3 \pm Pa_3$ (nc/mg)
11	0.083	7,500	97 ± 1.7
	0.5	19,300	250 ± 4.5
	1.0	16,600	248 ± 4.4
	1.5	14,750	235 ± 4.2
	2.0	11,660	186 ± 3.3
	2.5	8,800	140 ± 2.5
		$\rho_{35} = 62.7$	
12	0.083	2,350	37.1 ± 0.8
	0.5	10,300	163 ± 4
	1.0	12,300	195 ± 4.3
	1.5	12,500	198 ± 4.4
	2.0	11,200	178 ± 3.9
	2.5	7,000	111 ± 2.4
		$\rho_{35} = 63.2$	
15	0.083		1,565 ± 40
	0.5		793 ± 10
	1.0		368 ± 4
	1.5		156 ± 4
	2.0		50 ± 4

TABLE C-5 (Continued)

Compartment 3

Run No.	Elapsed Time (hr)	Rate of Change of Activity dR_5/dt (nc/hr)	Specific Activity $a_3 \pm \rho_{a_3}$ (nc/mg)
13	0.083	800	15.7 ± 0.3
	0.5	3,800	75 ± 2
	1.0	6,000	118 ± 2.5
	1.5	6,100	120 ± 2.5
	2.0	5,750	113 ± 2.4
	2.5	5,100	110 ± 2.1
		$\rho_{35} = 50.9$	
10	0.083	1,000	25 ± 0.6
	0.5	3,000	75 ± 2
	1.0	4,200	105 ± 2
	1.5	4,700	118 ± 2.6
	2.0	4,800	121 ± 2.7
	2.5	4,600	115 ± 2.5
		$\rho_{35} = 39.8$	

TABLE C-6

Peripheral Carbon Transfer Rates and Associated Standard Deviation

Carbon Transfer Rate ρ_{ij}	Dilution Factor D (hr^{-1})	Run No.	ρ_{ij} and Associated Standard Deviation (mg/hr)
ρ_{61}	0.42	11	165 ± 2.4
	0.50	12	200 ± 3.7
	0.51	15	203 ± 3.7
	0.54	13	222 ± 4.2
	0.59	10	236 ± 4.4
ρ_{14}	0.42	11	5.8 ± 0.3
	0.50	12	42.6 ± 1.3
	0.51	15	53.7 ± 0.8
	0.54	13	108 ± 1.0
	0.59	10	161 ± 1.6
ρ_{24}	0.42	11	74.1 ± 1.0
	0.50	12	80.2 ± 1.4
	0.51	15	75.5 ± 0.8
	0.54	13	56.6 ± 1.0
	0.59	10	32.3 ± 2.2
ρ_{34}	0.42	11	11.1 ± 0.4
	0.50	12	8.6 ± 0.3
	0.51	15	8.2 ± 0.3
	0.54	13	6.5 ± 0.3
	0.59	10	2.9 ± 0.2
ρ_{35}	0.42	11	74 ± 2.2
	0.50	12	68.4 ± 2.7
	0.51	15	65.6 ± 2.6
	0.54	13	50.9 ± 2.0
	0.59	10	39.8 ± 2.0

TABLE C-7

Cells to Organic Substrate (ρ_{21}) Carbon Transfer Rate with Associated Probable Error

Run No.	Elapsed Time (hr)	Slope and Percent Error (nc/hr)	Transfer Rate ρ_{21} (mg/hr)	Calculated Standard Deviation
11	1.5	- 800(1)	35.4	2.0
	2.0	- 333(1)	37.8	2.4
	2.5	- 167(10)	37.5	2.8
12	0.5	-63,500(5)	28.1	14.7
	1.0	- 6,500(1)	33.1	9.0
	1.5	- 1,500(1)	19.3	6.2
	2.0	- 1,500(1)	20.3	4.0
	2.5	- 500(10)	16.4	4.4
13	0.083	-70,000(10)	46.5	46.5
	0.5	-42,500(5)	40.0	37.0
	1.5	-14,700(1)	42.0	11.0
	2.0	- 8,700(1)	41.4	6.4
	2.5	- 4,500(10)	44.0	24.0
10	0.5	-45,600(5)	46.0	53.0
	1.0	-29,000(2)	74.7	119.0
	1.5	-18,000(1)	76.6	22.2
	2.0	-11,640(1)	61.9	16.3
	2.5	- 6,800(10)	51.6	23.7

TABLE C-8

Cells to Inorganic (ρ_{23}) Carbon Transfer Rate with Associated Probable Error

Run No.	Elapsed Time (hr)	Slope and Percent Error (nc/hr)	Transfer Rate ρ_{23} (mg/hr)	Calculated Standard Deviation
11	0.5	1,400(5)	104	59
	1.0	- 1,200(2)	86	44
	1.5	- 1,400(1)	99	47
	2.0	- 2,100(1)	89	21
12	0.5	1,900(5)	87	24
	1.0	1,000(2)	86	40
	1.5	- 100(1)	83	163
	2.0	- 750(1)	80	58
15	0.083	-120,000(10)	77	12
	0.5	- 50,000(10)	65	10
	1.0	- 25,000(5)	73	5.5
	1.5	- 11,200(1)	84	3.6
	2.0	- 2,800(10)	93	22
13	0.083	1,800(10)	65	9.6
	0.5	925(10)	76	22
	1.5	- 117(1)	65	158
	2.0	- 290(1)	66	62
	2.5	- 330(10)	67	50
10	0.083	1,200(10)	45.8	8
	0.5	425(5)	44.3	21
	1.0	240(2)	40.7	42
	1.5	220(1)	47.8	71
	2.0	- 160(1)	29	34
	2.5	- 240(10)	44.4	52

TABLE C-9

Adjusted Cells to Organic Substrate (ρ_{21}) Carbon Transfer Rate

D (hr^{-1})	$\rho_{21} \pm \sigma$ (mg/hr)	90% Confidence Limits (mg/hr)	Raw Mean (mg/hr)
0.42	36.6 ± 1.3	± 2.2	36.9
0.50	21.9 ± 2.5	± 2.4	23.4
0.51	25.8 ± 12		
0.54	41.6 ± 5.3	± 5.0	41.9
0.59	61.7 ± 10.6	± 10.0	62.2

TABLE C-10

Adjusted Cells to Inorganic (ρ_{23}) Carbon Transfer Rate

D (hr^{-1})	$\rho_{23} \pm \sigma$ (mg/hr)	90% Confidence Limits (mg/hr)	Raw Mean (mg/hr)
0.42	91.2 ± 16.7	± 19.7	93.4
0.50	86.1 ± 19.6	± 23.0	84.2
0.51	80.8 ± 2.8	± 2.6	78.4
0.54	65.2 ± 8.5	± 8.1	67.1
0.59	44.8 ± 7.1	± 5.8	42.0

TABLE C-11

Organic Substrate to Cell (ρ_{12}) and
Inorganic to Cell (ρ_{32}) Carbon Transfer Rates

D (hr^{-1})	$\rho_{12} \pm \sigma$ (mg/hr)	$\rho_{32} \pm \sigma$ (mg/hr)
0.42	195.8 ± 2.8	6.1 ± 3.3
0.50	179.3 ± 4.6	9.1 ± 4.6
0.51	174.1 ± 12.5	7.0 ± 3.0
0.54	155.6 ± 5.9	7.8 ± 4.7

TABLE C-12

Balance Equation (5-9) - Run 10

Elapsed Time (hr)	$\frac{dR_1}{dt}$ (nc/hr)	$-\rho_{14}a_1$ (nc/hr)	$-\rho_{24}a_2$ (nc/hr)	$-\rho_{345}a_3$ (nc/hr)
0.083	-49,000 -49,000	-48,400 -50,300	- 900	-1,000
0.5	-32,500 -32,500	-23,800 -32,800	-1,500	-3,000
1.0	-25,700 -25,700	-19,300 -25,500	-2,000	-4,200
1.5	-21,000 -21,000	-14,100 -21,000	-2,200	-4,700
2.0	-16,000 -16,000	-10,100 -16,900	-2,000	-4,800
2.5	-12,000 -12,000	- 6,900 -13,400	-1,900	-4,600

TABLE C-13

Balance Equation (5-9) - Run 11

Elapsed Time (hr)	$\frac{dR_i}{dt}$ (nc/hr)	$-\rho_{14}a_1$ (nc/hr)	$-\rho_{24}a_2$ (nc/hr)	$-\rho_{345}a_3$ (nc/hr)
0.083	-30,000	- 8,500	- 8,700	- 8,200
	-30,000	-25,400		
0.5	-33,000	- 1,200	-10,900	-21,200
	-33,000	-33,300		
1.0	-30,000		- 9,700	-21,000
	-30,000	-30,700		
1.5	-20,000		- 9,100	-19,000
	-20,000	-28,100		
2.0	-14,000		- 6,700	-15,000
	-14,000	-21,700		
2.5	-12,000		- 5,600	-11,000
	-12,000	-16,600		

TABLE C-14

Balance Equation (5-9) - Run 12

Elapsed Time (hr)	$\frac{dR_1}{dt}$ (nc/hr)	$-\rho_{14}a_1$ (nc/hr)	$-\rho_{24}a_2$ (nc/hr)	$-\rho_{345}a_3$ (nc/hr)
0.083	-30,000 -30,000	-23,800 -30,018	-3,368	- 2,850
0.5	-33,500 -33,500	-12,800 -33,600	-8,341	-12,500
1.0	-28,000 -28,000	- 4,560 -28,900	-9,460	-15,000
1.5	-18,400 -18,400	- 2,000 -26,000	-8,800	-15,200
2.0	-17,500 -17,500	- 937 -22,100	-7,540	-13,700
2.5	- 8,000 - 8,000	- 500 -14,600	-5,600	- 8,500

TABLE C-15

Balance Equation (5-9) - Run 13

Elapsed Time (hr)	$\frac{dR_1}{dt}$ (nc/hr)	$-\rho_{14}a_1$ (nc/hr)	$-\rho_{24}a_2$ (nc/hr)	$-\rho_{345}a_3$ (nc/hr)
0.083	-32,000 -32,000	-28,900 -31,100	-1,350	- 900
0.5	-25,000 -25,000	-18,900 -25,900	-2,700	-4,300
1.0	-22,100 -22,100	-13,300 -23,400	-3,400	-6,700
1.5	-18,000 -18,000	- 8,000 -18,600	-3,700	-6,900
2.0	-11,500 -11,500	- 5,400 15,300	-3,400	-6,500
2.5	-10,300 -10,300	- 3,500 12,800	-3,000	-6,300

TABLE C-16

Balance Equation (5-9) - Run 15

Elapsed Time (hr)	$\frac{dR_1}{dt}$ (nc/hr)	$-\rho_{14}a_1$ (nc/hr)	$-\rho_{24}a_2$ (nc/hr)	$-\rho_{345}a_3$ (nc/hr)
0.083	-120,000		- 300	-115,800
	-120,000	-116,000		
0.5	- 50,000		- 900	- 58,600
	- 50,000	- 59,500		
1.0	- 27,000		-1,000	- 27,200
	- 27,000	- 28,000		
1.5	- 11,600		-1,000	- 11,500
	- 11,600	- 12,500		
2.0	- 3,000		- 800	- 3,700
	- 3,000	- 4,500		

APPENDIX D

SUGGESTED FUTURE WORK

Suggested Future Work

The results of this research project suggest several areas of future work. Because of the short-comings of this research, all future work should include the determination of specific substrate as well as gross substrate.

1. It would be desirable to use the transfer rate technique to study other organisms and substrates to determine the degree to which generalizations can be made toward a kinetic model. Both soluble and insoluble substrates should be utilized in these studies.

2. Studies should be made using radioactive cultures fed no radioactive substrate in an effort to evaluate the heterogenous metabolism coefficient relationship to process loading.

3. The transfer rate technique should be applied to non-steady state systems (batch) for both soluble and insoluble substrates.

4. Since the compartmentalization is arbitrary in the transfer rate technique, the model can be extended to include other parameters than carbon such as organic compounds or inorganic elements.

5. The transfer rate approach could be used to study the kinetics of algae growth as they effect the problem of eutrophication.

6. The results suggest that the transfer rate technique might apply to the quantification of the inviability effect by use of carbon-14-dioxide.

APPENDIX E

PROJECT PUBLICATIONS

Publications:

Discussion by D. R. Washington, L. J. Hetling and S. S. Rao of "The Mathematics of Complete-Mixing Activated Sludge," R. E. McKinney, *Jr. of Sanitary Engineering Division of the ASCE*, *89*, SA1 (January 1963)

S. S. Rao, L. J. Hetling and D. R. Washington, "Spot-plate Technique for Measuring Bacterial Densities," *Indian Journal of Experimental Biology* (April 1963)

S. S. Rao, D. R. Washington and L. J. Hetling, "Percent Viabilities - A Measure of Activity," *Waste Engineering*, (December 1963)

D. R. Washington, "Theory of Extended Aeration Activated Sludge," *Gas Vent* - *New York Water Pollution Control Association Journal*, (December 1964)

S. S. Rao, L. J. Hetling and D. R. Washington, "Correlation of Percent Viabilities," *Naturwissenschaften*, *51*, No. 3, 70, (1964)

D. R. Washington, "Kinetics of Extended Aeration," *Proceedings of 13th Southern Municipal and Industrial Waste Conference*, April 16 and 17, 1964

E. J. Martin and D. R. Washington, "Kinetics of Steady-State Bacterial Culture. I - Mathematical Model," *Proceedings of the 19th Purdue Industrial Waste Conference*, May 1963

L. J. Hetling, D. R. Washington and S. S. Rao, "Kinetics of the Steady-State Bacterial Culture. II - Variations in the Synthesis," *Proceedings of the 19th Purdue Industrial Waste Conference*, May 1963

Awaiting Publications:

L. J. Hetling and D. R. Washington, "Kinetics of Steady-State Bacterial Culture. III - Growth Rate," Proceedings of the 20th Purdue Industrial Waste Conference, May 1964

E. J. Martin and D. R. Washington, "Kinetics of Steady-State Bacterial Culture. IV - Transfer Rates," Proceedings of the 20th Purdue Industrial Waste Conference, May 1964

D. R. Washington, E. J. Martin, and L. J. Hetling, "Yield in Continuous Aerobic Bacterial Fermentation," ACS Symposium on "Scale-up Problems in the Fermentation Industry," Sept. 1965, Bio-engineering and Technology